The Microbial Populations Of The Intertidal Zone Of Two Sandy Beaches

Thesis

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THE MICROBIAL POPULATIONS OF THE INTERTIDAL ZONE OF TWO SANDY BEACHES

A thesis offered for the degree of Master of Philosophy in the discipline of Biology

by

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THE MICROBIAL POPULATIONS OF THE INTERTIDAL ZONE OF TWO SANDY BEACHES

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Abstract:

This study was based on an examination of the bacterial and fungal populations in the intertidal zone of two sandy beaches. The two beaches were near Hartlepool, Cleveland, and one was selected as being heavily polluted by a major sewage outfall whilst the other appeared to be relatively unpolluted. Several physical and chemical characteristics of the two beaches were also assessed.

The results show that there were resident populations of heterotrophic bacteria present in both beaches. These populations consisted principally of species of Pseudomonas. Fungi were also isolated from both sites. Most of the genera recorded are normally regarded as terrestial. There was an inverse correlation between the numbers of bacteria and fungi in both beaches. No major qualitative or quantitative difference was found between the microbiology of the polluted and the non-polluted beach. Human faecal bacteria were not found in either sediment.

Experiments carried out on the effects of adsorption in these intertidal sands exphasized the importance of this phenomenon in the microbial ecology of these substrates. Desiccation was shown to profoundly change the bacterial flora of sand but water loss was considered to be minimal in the zones subject to twice daily inundation.

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I also state that I am willing that this work may be made available and/or photocopied at the discretion of the librarian of the Open University. If feasible I would be grateful if such loan or copying could be notified to me.

I wish to acknowledge the debt of gratitude I owe to the large number of people without whose support or assistance this thesis could never have been completed.

I must first thank the District Management Team of the Hartlepool Health District of Cleveland Area Health Authority who gave me permission to use the facilities of the laboratories at the General Hospital, Hartlepool to carry out the practical work contained in the thesis.

The instruction, guidance and encouragement of my external supervisor, Dr. C.H. Dickinson of the Department of Plant Biology, University of Newcastle Upon Tyne, was given unstintingly over five years of study and his help was essential to the work's completion.

Dr. Mary Bell of the Open University was always helpful in her supervision of the study programme.

Mr. R. Allen Reese of the University of Sheffield Computing Services carried out the statistical analysis of the microbiological data and gave advice on statistical aspects generally. However, any errors that may be present will certainly be attributable to me and the conclusions drawn from his analysis are my own.

Mrs. H. Monk undertook the considerable task of translating into typescript the several drafts of this thesis and also of a dissertation which formed part of my preparation for this work.

My colleagues in the Department of Pathology of Hartlepool General Hospital have shown great forbearance with my desire to discuss problems arising in the work and have not complained at the frequent crunch of sand beneath their feet.

Finally I must acknowledge the fact that my wife and family have never grumbled when their needs have been neglected in order to allow work on this thesis to progress.

TABLES

Page No.

FIGURES

A. INTRODUCTION

The edge of any body of water has always been a source of attraction to man. It represents a line of demarcation between his own environment and one that appears foreign to him. Water movement, caused by currents, surface disturbance by wind, and tidal effects, acts as a means of transport for anything that enters or exists in it. This movement also deposits an infinite variety of materials along the water's edge.

In the case of the sea man's fascination is at its greatest; it has the constant movement of the waves, it is visually limitless and the materials deposited along its edge may have travelled many thousands of kilometres. This jetsam has always provided coastal man with materials he needed to exist and has given rise to the age-old occupation of beach-combing.

The edge of the sea is, for similar reasons, an area of great biological interest. It represents the line at which there is a change from the aquatic to the terrestial and from the saline to the non-saline and, therefore, physically represents that point at which a fundamental evolutionary change is believed to have occurred.

This line is not, of course, a static one. The semi-diurnal rise and fall of the tide results in the periodic emersion and submersion of an intertidal zone, the width of which depends upon a variety of factors including the slope of the shore and the extent of the rise and fall of the tides. The physical features of such a zone are immensely complex and vary not only from coast to coast but also within comparatively small distances on any one shore (Newell, 1970).

The intertidal zone may be either rocky or sedimentary. On the rocky shore any deposited material is swept away by ebbing tides, leaving only that trapped in cracks and fissures in the rock. In this situation the rocky edge of the land may be broken down by marine erosion to form a pebbly beach.

On a sedimentary shore particulate material is deposited and much of it remains when successive tides recede. Current changes, dredging and other factors can rapidly convert a sedimentary shore to a rocky one and vice versa. However, even on a 'permanent' sedimentary shore a dynamic state exists and Ranwell (1972) has pointed out that under relatively calm conditions the level of the shore may alter by as much as 0.3 m within as little as 12 h.

Ranwell (1972) has also stated that the principal source of inorganic sediment on such shores is the aerial or marine erosion of the land surface (and to a lesser extent the sea bed) mainly during periods of high wind or heavy rainfall; subsequent deposition of this material occurring in calm weather conditions.

The nature of the deposited material will depend upon its ultimate source. For example, in Britain to the North and West of a line from the Tees to the Exe hard rocks predominate and this is reflected in the predominance of coarse sediments which form a discontinuous band of various thickness and width along the shore line of this region.

However, where the source of sediment is soft rock, soil or clay then a muddy shore may result and, where the physical features of the shore are appropriate, mud-flats and salt marshes are formed. Such conditions will often occur around an estuary.

The inorganic material deposited in the intertidal zone is most often the loose, non-cohesive, granular material which we call sand. The size limits, which define sand, vary according to different authors but Ritchie and Mather (1969) have given a range of 100 μm to 1100 μm.

Such sand is usually of mixed geological origin and may be quartz, carbonate, or oditic sand but the most common sand is siliceous (Pettijohn, Potter and Siever, 1972).

These intertidal sediments have been studied with some thoroughness by geologists, oceanographers and zoologists but have been relatively neglected by microbiologists. The study of their microbiology obviously

required knowledge of marine microbiology and this subject was neglected, until relatively recently, by all but a devoted few. Indeed Zobell, who can perhaps be described as the 'father' of marine bacteriology felt the need, only thirty years ago, to appeal to bacteriologists to take an interest in the sea (Zobell, 1946). Since that time there has been an exponential increase in the work being carried out by microbiologists in the marine milieu. This is, in part, a reflection of the general increase in the number of scientists but is also due to a growing realisation of the biological and economic importance of the sea.

The majority of the microbiological work that has been carried out has been on seawater itself; less has been done on the marine sediments and very little on the relationships between the microbial flora of the water and the sediments.

The sediments deposited along the edge of the sea have been studied microbiologically by very few workers. Some of these have been zoologists trying to find the answers to problems concerning the nutrition and settlement of the animals present in the intertidal zone (e.g. Meadows, 1964; Wilson, 1948 - 1955; Gray, 1966). The neglect by bacteriologists and mycologists is strange because these intertidal deposits are easily sampled and an examination of their microbial populations could well provide information of great value to the general understanding of sediment/water relationships.

B. PREVIOUS WORK

One of the earliest papers on the bacterial populations of sand from the intertidal zone was that of Pearse, Humm and Wharton (1942) who observed by direct examination that bacteria appeared to be firmly fixed to the surface of the sand grains, and then, using viable counting methods they estimated that the bacterial population varied from $5 \times 10^3 - 1250 \times 10^3$/g wet sand. Their mean value for mid-tide line samples was 110×10^3/g wet sand and the highest counts they obtained were from the 'encrusting film' of the surface sand ($5 \times 10^4 - 5 \times 10^6$/g wet sand).

Stimulated by some earlier work on substrate selection by Corophium Meadows and Anderson (1968) carried out some interesting work on the colonisation of sand particles by bacteria and they were able to produce excellent photographs showing bacteria attached to the grains. Vigorous agitation of the sand in various solutions removed varying numbers of these bacteria from the grains (Anderson and Meadows 1965, 1969). Counting the released bacteria directly in a haemacytometer chamber they obtained very high results ranging from $140 \times 10^6 - 1183 \times 10^6$/g dry, washed sand and they claimed that similar results could be obtained by measuring the optical density of their supernatants as a measure of bacterial numbers. Further calculations showed that bacterial numbers could be as high as 259×10^3/mm^2 surface area of sand grains. Viable counts gave figures varying from $2.6 \times 10^3 - 241 \times 10^3$/g dry, washed sand which they converted to 0.2 - 40/mm^2 surface area. Thus by using direct methods they estimated the bacterial population to be up to 6.5×10^3 times greater than that indicated by a cultural method. In these studies samples were taken at random from 12 beaches with particle sizes varying from 180 - 520 µm but no relationship was found between particle size and bacterial count.

Grey (1966), in laboratory experiments, found that sterile sand inoculated with natural sand could support bacterial populations,

estimated by viable counts, as high as 18 x 10^6/g sand. He was also able to show that many bacteria were able to survive in wet sand at 40°C but were killed at 50°C. His findings suggested that sand was attractive to Protodrilus symbioticus not because of the presence of bacteria as such, but because of the organic film these bacteria produced on the grains.

Khiyama and Hakenson (1973) have enumerated sand beach bacteria using both direct and viable counts. The number of bacteria estimated by the direct method was one thousand times greater than the numbers grown on culture media. Viable counts carried out on distilled water based medium gave results ranging from 0.7 x 10^2 - 2.7 x 10^3, and on seawater based medium the range was 7.8 x 10^2 - 4.0 x 10^4 per g of wet sand. They also carried out a series of biochemical tests on over 350 isolates and found that about 70% were gram negative rods and that about 60% of these were non-fermentative. Over half their isolates were indole-positive and they suggested that fermentative ability together with indole production indicated a symbiotic association with metazoans resident in the sand.

Dale (1974) has made a study of the factors affecting the distribution of bacteria in intertidal sediments. He separated bacteria from sand particles by homogenisation and after filtering, stained the bacteria with acridine orange and counted them by fluorescent microscopy. With this technique he found numbers ranging from 1.77 x 10^3 - 9.97 x 10^9/g of dry sediment. He showed a clear inverse relationship between numbers and grain size and also found a strong correlation between carbon and nitrogen levels and bacterial numbers and mean grain size. He suggested, therefore, that there were good reasons for considering the area available on particle surfaces as the key property affecting both bacterial numbers and the levels of organic carbon and nitrogen.

Rheinheimer (1974) stated that the highest numbers of bacteria and fungi are almost always found on the beach surface and that below the surface these numbers may be reduced to a few per cent of the surface total. He concluded that sandy beaches are colonised by several hundred

thousand or millions bacteria per cm^3 and that under jetsam their numbers may rise to more than 20 million.

Perkins (1974) in studies of the Firth of Clyde and of the sands of Moray, Inverness-shire showed sand-grains to be colonised by bacteria, blue-green algae and diatoms. He asserted that the microbial flora may alter only slightly to depths of 15cm below the sediment surface or it may change within a few millimetres. He also found that whilst the flora was sparse towards high-water mark there was little difference between the sands of the lower shore and the sub-littoral.

Pugh, Andrews, Gibbs, Davis and Floodgate (1974) counted the bacteria in two beaches in North Wales and found that on one mean numbers of viable bacterial units during a two year period in the 0 - 1 cm horizon varied from 66×10^3/ml - 95×10^6/ml of associated water whilst the second beach gave counts ranging from 195×10^3 - 9×10^6/ml. The overall pattern shown by their figures showed that numbers decreased with depth and also down the intertidal zone.

Andrews, Floodgate and Pugh (1976), using a model beach, were able to demonstrate that bacterial numbers were highest near the high and low water marks and reached a minimum in between on the beach slope. The numbers decreased with depth in their sand profile and varied little with time. These workers also examined the characteristics of 42 of their isolates. They found that the majority were gram negative rods and tentatively identified 16 of them as Pseudomonas.

Although there have been several reports of isolation of fungi from sand dunes (e.g. Webley, Eastwood, Gimingham, 1952; Saîto, 1955; Dickinson and Kent, 1972) and from salt marshes (e.g. Elliott, 1930; Saîto, 1955; Pugh, 1962) there has been very little work done on recovering fungi from the intertidal zone of sandy beaches. Some studies have been carried out by Brown (1957, 1958 a, b) and Nicot (1958 a, c) as part of studies of sand dunes. Brown isolated fungi from areas of the beach which were subject to regular inundation by the sea but the use of the term 'open sand'

makes it uncertain exactly from where her samples were collected. Nicot isolated fungi from the upper part of the beach which was subject to the effects of salt spray or immersion by the very highest tides. Both Brown and Nicot found that fungi were scanty in the intertidal zone. Brown (1958 b) reported that 61% of her soil plates from the 'open sand of the foreshore' were sterile but she found small 'oases' where fungi were present which were coincident with pockets of organic material. The most common genera she isolated from open sand were Penicillium and Cladosporium.

C. OBJECTIVES

(1) It was thought that the reporting of microbiological findings alone would have much less value if no information was presented on the physical and chemical characteristics of the substrate. A subsidiary aim has, therefore, been to provide this information.

(2) As already stated, the intertidal zone may consist of deposits which vary from large pebbles to fine muds. This study has been restricted to the examination of the 'sand' beaches.

(3) The main aim of the work has been to examine the populations of bacteria and fungi in intertidal zones and where possible to interrelate the findings on both. This does not seem to have been attempted before.

(4) Ecologists generally have shown that population size more or less reflects the nutritional state of an environment. However, as Munro (1975) has pointed out the microbial ecologist cannot easily draw such a conclusion because of the enormous versatility of microbes in producing dormant stages whenever environmental stages are unfavourable. In this work this fact has been kept constantly in mind and steps have been taken to restrict the study, as far as this was possible, to those organisms actually growing in the sand at the time the samples were collected.

(5) Most previous workers have examined beaches which were apparently free from pollution. A deliberate decision was made to select, as one of the two beaches to be sampled for this work, a beach that was grossly polluted.

A second objective has, therefore, been to assess the effects of pollution on the microbiology of sandy sediments by counting both bacteria and fungi, on several occasions, from samples of sand collected within the intertidal zone of the two beaches. Both direct and viable counting methods have been attempted.

A qualitative assessment of the microbial populations was also made and the significance of the findings have been assessed in the light of the information gained about the intertidal samples examined.

Both beaches were examined for the presence of human faecal flora in order to estimate the extent to which these survived in the environment.

(6) It became obvious during the early work that adsorption of microbes to particles was probably of cardinal importance in the ecology of beaches. Experiments were, therefore, devised to clarify this phenomenon.

(7) Having achieved these objectives the aim has been to correlate the findings with those of other workers and to thus provide a description of the microbial ecology of sandy beaches.

D. MATERIALS

SITES

Two beaches were chosen for sampling, both of which are within easy reach of the base laboratory, which is in the town of Hartlepool, Cleveland, in North East England. This made it possible for samples to be transported quickly for immediate examination. One beach, at Crimdon, is about 4 km North of Hartlepool (Nat. Grid. Ref. NZ 489370) and the other, Seaton Carew, is on the Southern edge of the town (Nat. Grid. Ref. NZ 519519).

These beaches face onto the North Sea and are within 15 km of the estuary of the River Tees which discharges into Hartlepool Bay and is one of the most polluted rivers in England. The Third Report of the Royal Commission on Environmental Pollution (1972) stated that 4×10^8 gallons of trade wastes were being discharged daily into the Tees and it's estuary. Half of this came mainly from the chemical industry and half was in the form of cooling water. On this stretch of coastline 9.5×10^6 gallons of untreated sewage were being discharged daily into the sea, half of this being of trade origin. North of these beaches 2.5×10^6 tons of colliery waste were being tipped directly into the sea. Sufficient of the coal fraction of this waste is washed back onto beaches in the Hartlepool area to support a small, but thriving, industry of seacoal-gatherers.

The Crimdon beach is, however, an aesthetically pleasant one, with a slight inward curve and a moderately steep slope. It is backed by an early dune system and then by a cliff which varies in height from 40 - 100 m and is composed of boulder clay and limestone. Some 30 - 40 m of this beach are above high water mark (HWM). At the Southern end a 'dene' abutts onto the beach (a dene is the local name for a wooded valley running from well inland to the sea) and a freshwater stream running from

it crosses the beach in a shallow channel. The cliff tops are used by a caravan camp and in summer the beach has many human visitors. Pollution with sea-coal occurs but due to prevailing currents not so heavily as on the beaches of Hartlepool itself.

Seaton Carew has a narrow, almost flat, beach of shallow sand with occasional outcroppings of rock. At its Northern end, known as Newburn, a sewage outfall runs 100 m out to sea and a storm drain discharges directly onto the beach. The outfall discharges 95% domestic sewage which is untreated apart from large-mesh filters to screen out larger objects. During 1976 4.5×10^6 gallons were discharged daily from this outfall (estimated figure from local water authority).

Dredging of the Tees and new defence-works at Hartlepool are causing loss of the finer sand from this beach.

A sloping 12 m high defence wall backs the beach and supports the promenade and all but very low tides submerge the whole beach. There are few pleasure-visitors due to the outfall but sea-coal gatherers are regular users.

The actual sampling sites are shown on the maps (fig. 1) and photographs (fig. 2). The site at Crimdon was some 200 m from the stream that crosses the beach at its Southern end. The Newburn site was 50 m from the sewage outfall. Points of sampling were kept constant by observation of fixed 'marks' - flagpole at Crimdon and a measured distance from the outfall at Newburn.

Fig. 1a

Sampling Sites

Fig. 1b SAMPLING SITES

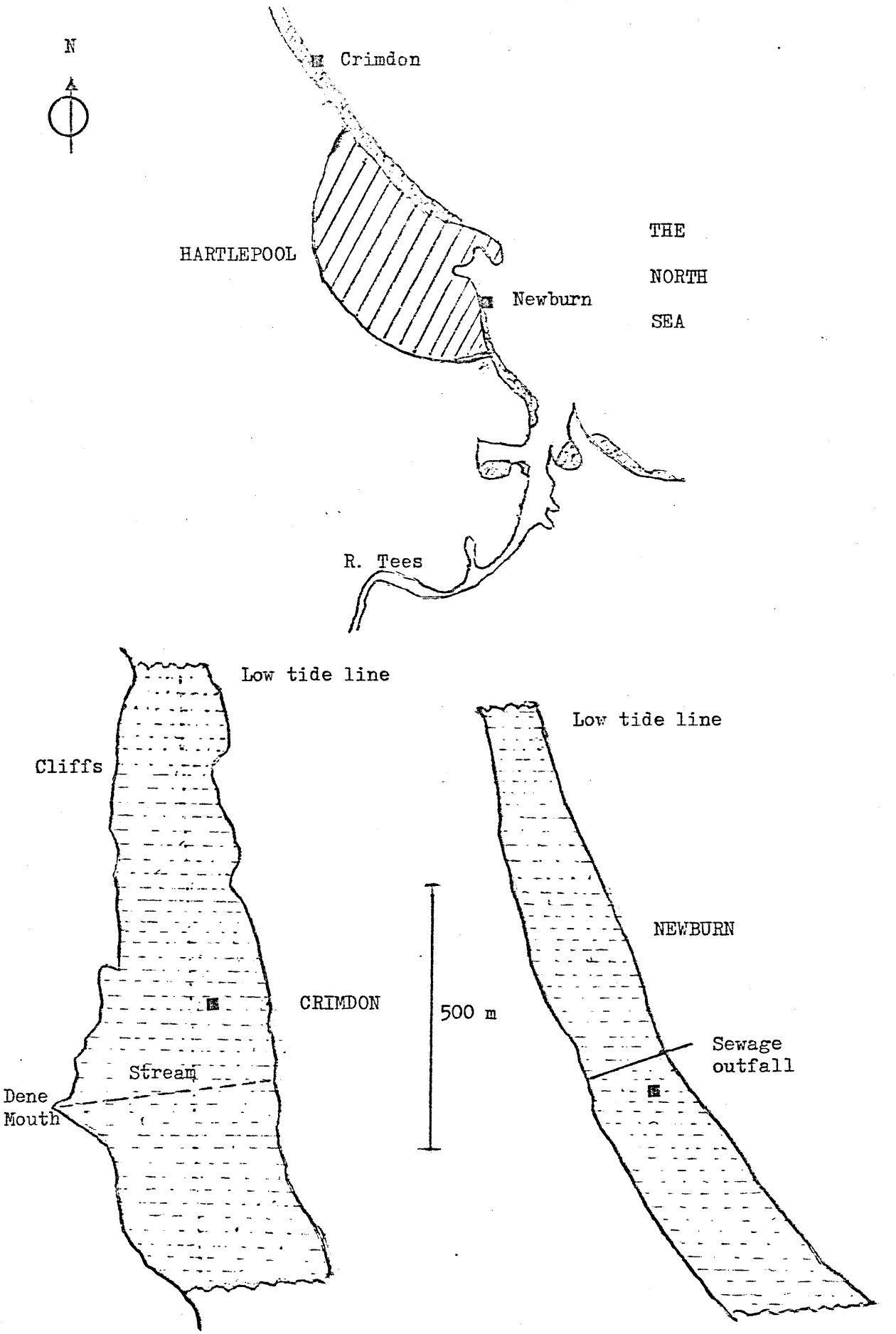


Fig. 2 SAMPLING SITES

Newburn (showing sewage outfall

Crimdon (★ indicates sampling area)

E. METHODS

i. COLLECTION OF SAMPLES

Pearse, Humm and Wharton (1942) and Zobell and Feltham (1942) reported that bacteria are most abundant in the upper layers of marine sediments and more recently Rheinheimer (1974) showed that highest numbers of bacteria and fungi are almost always found in the top few cm and are particularly abundant on the surface. All samples were therefore collected from the 0 - 1 cm depth of the beach.

For each sample an area 15 x 10 cm was scraped to 1 cm deep with a sterile spatula and the sand placed in a sterile, glass, screw-capped jar. When a series of samples were collected they were taken at 10 m intervals along the mid-tide line, starting from the sampling point indicated by the fixed mark and progressing southwards. For this purpose 'mid-tide line' was defined as a line equidistant from the HWM and the LWM of the particular day's tide. Pugh, Andrews, Gibbs, Davis and Woodgate (1974) sampling along a fixed transect of a beach obtained some anomalous results and found this was due to the fact that one of their sampling stations coincided with an old tidal strandline. They pointed out that this illustrated the inadvisability of using the mid-tide line as a means of obtaining a representative sampling of a whole beach transect. This criticism cannot be applied to the present study since it was not aimed at representing a transect but a strip of beach, within the intertidal zone and lying parallel to the sea's edge. Furthermore the mid-tide line, as defined for this work, was not a fixed line but one which varied according to tide-levels on the day of sampling.

It was not possible to sample both beaches on the same day as the subsequent work-load would have been too great and it was estimated that work carried out on each batch of samples could take up to three months to complete. It was clear, therefore, that temperature, weather and

season might differ greatly on each sampling occasion. Two courses were therefore possible. One was to attempt to obtain close matching of environmental conditions for the sampling occasions from each beach; the other to ignore environmental conditions and avoid bias by random selection of the beach to be sampled. The latter course was chosen and beach selection was by toss of a coin.

Similarly, no attempt was made to select particular tides, except that days were chosen when the mid-tide line was uncovered at a time which allowed subsequent culture work to be carried out immediately. The tides were, however, always such that the mid-tide line fell within a zone that was 'intertidal' when that term is defined as an area of beach which is covered by the sea at least once in every 24 h throughout the year.

Completely atypical sampling points were avoided e.g. where there was a substantial drift of sea-coal or cast-up seaweed. On no occasion had there been substantial human disturbance of the sampling area since the tide had receded. The Orimbon site had been chosen because it was some distance from the access steps around which visitors congregate. One sampling visit to Newburn coincided with the activities of the sea-coal gatherers and sampling was postponed.

Seawater samples were collected from the breaker zone by wading out and half-filling, by submersion, a sterile, glass, 5000 cm^3 bottle. These samples always contained particulate debris including fine sand.

Sand-free sea-coal samples were collected from a heavy drift using a sterile spatula.

At Newburn, in spite of large volumes of sewage being discharged near to the sampling site, there were only three occasions when inspection showed recognisable faecal deposits. On these occasions a single whole stool was placed into a sterile, glass jar for subsequent examination. Faeces was never seen at Orimbon.

ii. PHYSICAL ANALYSIS

a. Water Retaining Capacity

25g of air-dried sand were firmly packed into the barrel of a 20 cm^3 polypropylene syringe (Becton-Dickinson Plastipak) in the base of which had been placed a single layer of cotton gauze to prevent sand flowing through the nozzle. Cotton wool and glass wool had also been tried for this purpose but, unlike the cotton gauze, interfered with water flow.

Filtered seawater was then dripped slowly into the syringe from a 10 cm^3 pipette until the sand was saturated, as indicated by a drop falling from the nozzle of the syringe. At this end-point the volume of water which had been added was noted.

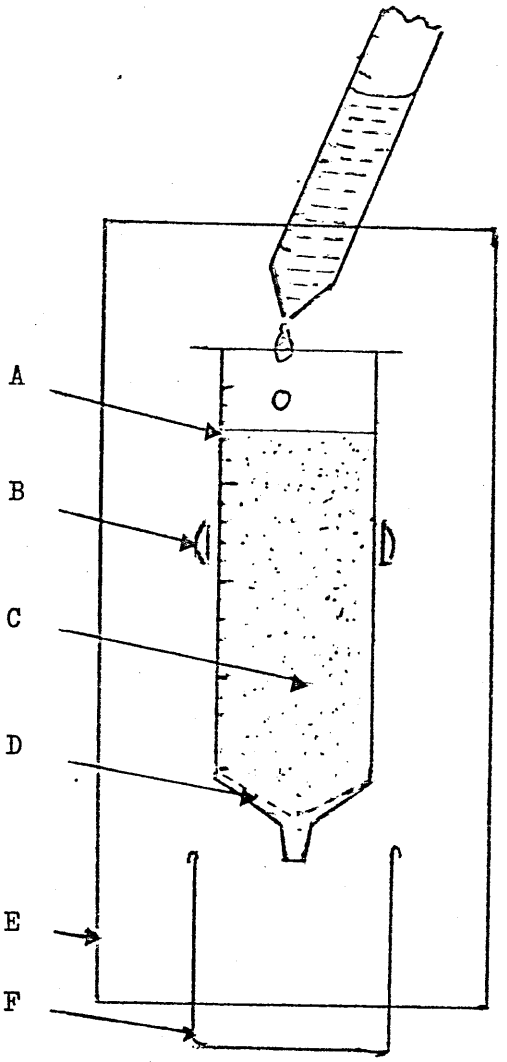
About 5 cm^3 of air were then forced through the sand using the syringe plunger. The volume of water expelled was measured and noted. (see fig. 3a for diagram of apparatus).

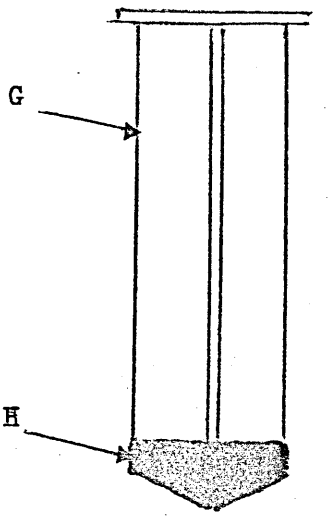
A wet sample of sand was allowed to stand in a screw-capped container in the refrigerator overnight. Any supernatant water was then drained off as completely as possible and a 25 g aliquot was placed into an open petri dish. This was allowed to stand at room temperature (22 - 24 °C) until the sand was dry as judged by the capacity of the individual grains to move easily when poured. The aliquot was reweighed and the difference noted.

b. Flow Rate

25 g of air-dried sand were packed into a syringe as described in ii.a. 10 cm^3 of filtered seawater were then pipetted quickly into the syringe and at the same moment an unobserved stopwatch started. The

Fig. 3a APPARATUS FOR ESTIMATING WATER RETAINING CAPACITY

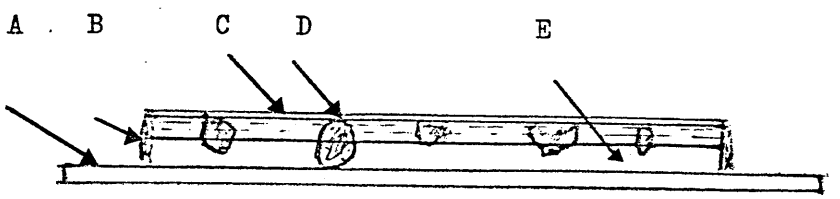




A. Syringe
B. Terry clip
C. Sand
D. Cotton gauze
E. Heavy wooden block
F. Catchpot
G. Syringe plunger
H. Heavy rubber tip

Fig. 3b METHOD USED FOR EXAMINING SAND GRAINS

(From Gray and Parkinson, 1968)



A) Microscope slide B) Varnish seal C) Coverslip

D) Sand grain E) Agar layer

water rapidly saturated the sand and dripped from the nozzle. As the last drop fell the watch was stopped and the time of flow-through was noted. This proved to be a reliable and clear-cut endpoint. Using the same column addition of 10 cm^3 volumes was repeated until flow-through times were obtained which were within 2 seconds of each other.

Still using the same column a series of 10 cm^3 volumes of deionised water were added. By testing these aliquots with silver nitrate solution as they were collected it had been found that at least 5 x 10 cm^3 volumes were needed to flush away all salinity, therefore at least 8 volumes of water were always used to obtain flow rates.

This whole process was then repeated using a new sand column prepared from the same sample but on this occasion deionised water was added first.

c. Water Content

Samples of surface sand were collected over a single tide cycle on each beach. First samples were collected immediately after the ebbing tide had exposed the sampling area and the last just before the flowing tide immersed it again. 25 g of each sample was then removed, dried at room temperature, as previously described, and reweighed.

d. Grain Size

10 samples of sand from each beach were collected. These were bulked and dried at 60 ^{o}C for 24 h. The dried sand was thoroughly mixed and split into four aliquots which were then sieved through square-mesh sieves to obtain proportions.

c. Morphology of Grains

Because of the varying size of sand grains and their relatively large volume it is difficult to observe them with the ordinary light microscope because of the limited depth of focus. Since the upper surface of larger grains is well above that of small ones it is also impossible to observe the small grains with the higher power objectives without grinding the lens into the preparation. Some workers (e.g. Brown, 1959) have used a metallurgical microscope to overcome this difficulty. Since such a microscope was not available several methods were tried and finally that described by Gray and Robinson (1968) was adopted and phase contrast microscopy was used to examine the preparation (see fig. 3b).

To obtain some knowledge of the sand content, 'differential' counts were carried out characterising the particles into the following classes as judged from their microscopic appearance:-

Siliceous grains

Shell fragments

Coal particles

Cellular organic fragments

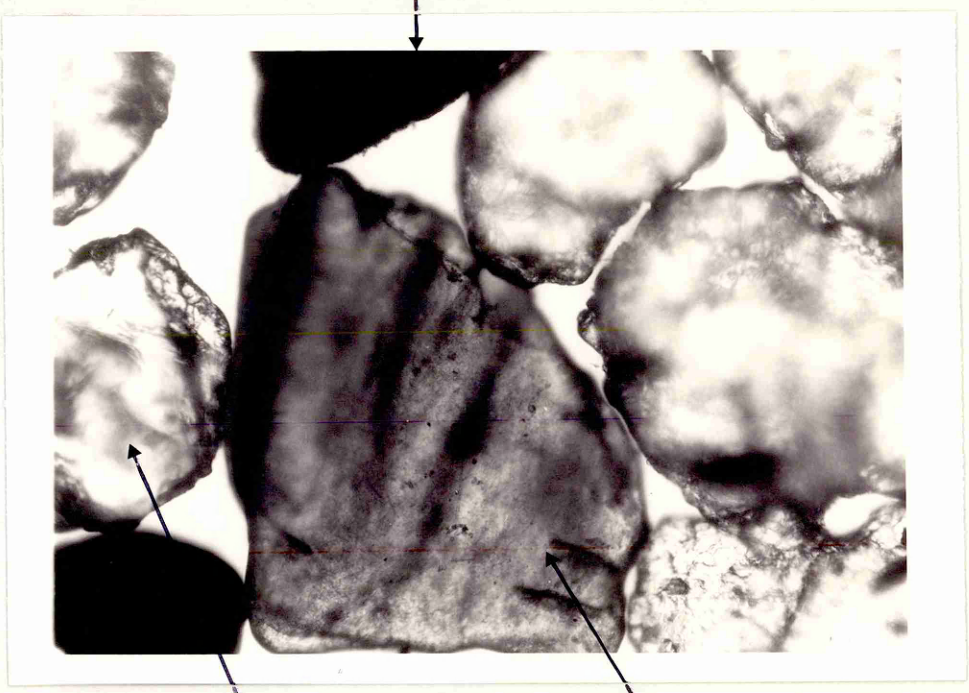
Other organic fragments (see photograph. fig. 4)

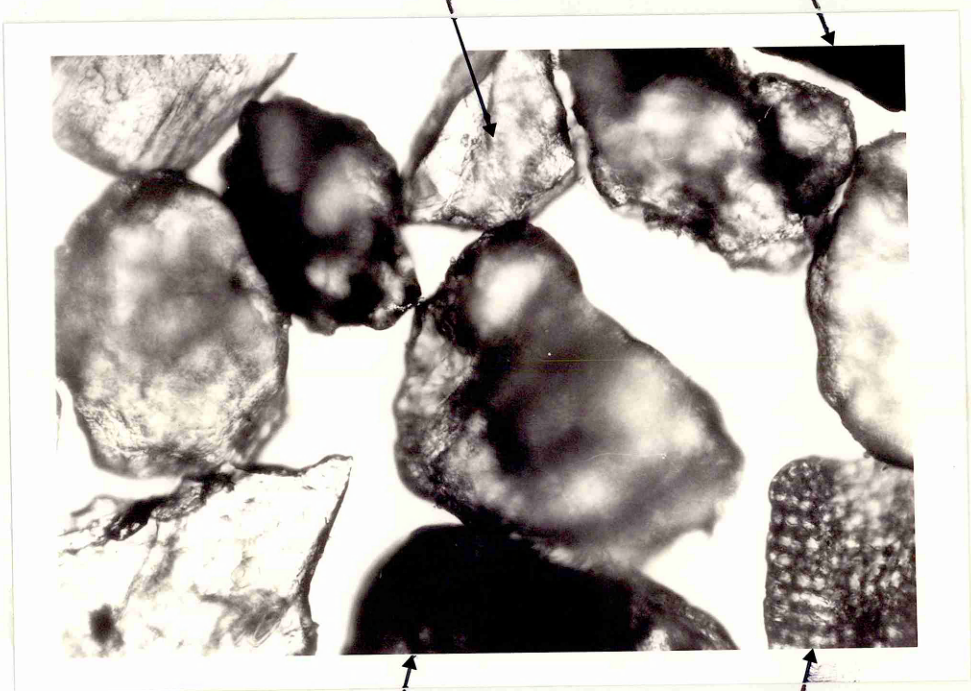
To obtain reasonable estimates 10 counts, each of 500 particles, were carried out on a thoroughly mixed bulk sample of air-dried sand from each beach.

Attempts were made to identify a pigmented deposit seen on and within the particles. First the grains were treated with a range of concentrations of sodium hydroxide and hydrochloric acid, at different temperatures, and were examined microscopically before and after treatment.

Subsequently grains were tested with an iron-detecting reagent 0.2% 2.2 dipyridyl in 6% aqueous acetic acid. The reagent was pipetted

Fig. 4. Different classes of particle seen in surface sediments





under the coverslip of a microscopic preparation and any reaction was noted.

iii. CHEMICAL ANALYSIS

a. Salinity

Measurements were carried out on the supernatant water resulting from allowing samples, collected soon after the tide had ebbed, to stand overnight at 4 - 6°C. The supernatant was removed and centrifuged until clear. Three volumes of deionized water were then added to one volume of supernatant and the sodium, potassium and chloride levels were measured using the Technicon autoanalyser. This is a continuous flow system and the analytical methods used were flame photometry for sodium and potassium and Skegg's modification of the method of Zall, Fisher and Garner (1956) for chloride. The height of the peaks recorded for the test samples was measured and compared with peak-heights given by a range of combined standards tested at the same time. Results were expressed in mmol/l.

Tests for bicarbonate and urea were carried out at the same time using the methods of Skeggs (1960) and Marsh, Fingerhut and Miller (1965) respectively.

b. Organic Carbon

The method devised by Walkley and Black and modified by Baker (1976) was used. The probe colorimeter, however, was replaced by an EEL long-cell absorptiometer.

10g of air-dried sand were placed into a 250 cm^3 Pyrex Erlenmeyer

flask and 10 cm^3 of approximately molar potassium dichromate added. The flask was swirled thoroughly to uniformly wet the sample. Then 20 cm^3 of concentrated A.R. sulphuric acid were added and the flask contents mixed well and left to cool for 1 h. 60 cm^3 of deionised water were poured in and mixed thoroughly before an aliquot was removed and centrifuged at 2500 r.p.m. for 15 min. The optical density of the supernatant was measured using a 625 filter and a 1 cm cell. The control contained reagents only.

The control reading was subtracted from those of the samples and these were then converted to mg C using a calibration curve prepared from standard A.R. sucrose solutions. Results were expressed as mg/kg sand.

c. Nitrogen

The semi-micro Kjeldahl titration method was used. Five samples of air-dried sand from each beach were finely ground and approximately 0.5 g aliquots weighed into Kjeldahl flasks. The sand was moistened with 1 cm^3 of distilled water and then 10 cm^3 of concentrated sulphuric acid and a catalyst tablet were added. This digestion mixture was brought slowly to boiling point and, when clear, boiled for a further 2 h.

After cooling, distilled water was cautiously added in approximately 20 cm^3 aliquots, gently mixed with the sediment and decanted, after settling, into a 100 cm^3 volumetric flask. The volume was finally made up accurately to 100 cm^3.

Distillation of this digest was carried out in a Buchi distillation apparatus, in 10 ml aliquots, after addition of 10 ml of 50% sodium hydroxide solution. The distillate was passed into 10 ml of boric acid with indicator.

Titration was carried out against 0.01 N hydrochloric acid and the level of nitrogen calculated taking into account the acid-factor and the

result of a blank titration.

iv. MICROBIOLOGICAL ANALYSIS

a) Direct Observation

(1) Fixed and unfixed preperations

The unfixed preparations used for the differential counts of sand particles were examined, using phase contrast, with a high power, dry objective. No quantitative assessment of bacteria or fungi was attempted at this stage.

The sand was then fixed. Meadows and Anderson (1968) had used Bouin's fixative and post-fixation with osmic acid subsequently storing the fixed sand in formalin. As samples fixed with formal-saline proved equally satisfactory this method was used throughout.

A knife-point of the fixed sand was added to about 1 cm^3 of the staining solution and allowed to stain (3 - 5 min for bacteria; 1 h for fungi). The stain was poured off the sedimented grains and they were then washed several times in water and mounted by the method of Gray and Parkinson (1968). Where more permanent preparations were required the grains were dehydrated quickly in ethanol, rinsed in xylene and mounted in a large enough volume of a resin mountant (Styrolite - Raymond A Lamb) to ensure a layer thick enough to embedd the largest grains. After hardening, these preparations could be examined with high power, oil-immersion objective.

Several staining solutions were tried including methylene blue, methyl violet and Ziehl-Neelsen's carbol - fuchsin solution for bacteria and phenolic aniline blue for fungi and bacteria.

(2) Agar block preparations

Sand was sprinkled onto the surface of a prepared plate of culture medium and then from selected areas of the plate, where grains were well spaced, squares of medium were cut out and mounted on a sterile microscope slide. A sterile coverslip was superimposed and pressure applied to force the grains into the agar. This preparation was placed into a moist chamber (prepared from a sterile petri dish containing a pledget of moist cotton wool) and incubated. The embedded grains were examined microscopically every 48 h for 2 - 3 weeks.

(3) Direct counts

Attempts were made to enumerate bacteria and fungi using two techniques. The first was that of Jones and Mollison (1948). The sand was repeatedly ground in a pestle and mortar with a known volume of 3% sodium chloride solution. 1.5% agar-agar solution was added to the resultant suspension and the mixture pipetted into a haemocytometer chamber and allowed to set to form an agar film of known thickness. This film was removed; floated onto a microscope slide; allowed to dry and stained. The organisms present in defined areas were counted and the number per g of soil calculated.

The second method tried was that described by Parkinson, Gray and Williams (1971). Here a film of a suspension obtained by blending was spread over a 1 cm^2 area of the slide and, after heat fixation, was suitably stained and the number of organisms in defined areas were counted.

b. Cultural Techniques

(1) Preparation of suspension

The first problem encountered involved removal of organisms from the particles. Inoculation of media with sand grains gave profuse and

mixed colonies close to the grains. Mixing grains with 3% sodium chloride solution followed by plating of the supernatant gave rise to only a few colonies.

The literature gives several methods which have been used to obtain maximum colony counts from sand, such as:-

Vigorous agitation of sand in a diluent using a reciprocating shaker (Anderson and Meadows, 1969)

Use of detergent solution as diluent (Gray, 1966, Anderson and Meadows, 1969)

Use of glycerol or sucrose solution as diluent (Meadows, 1964)

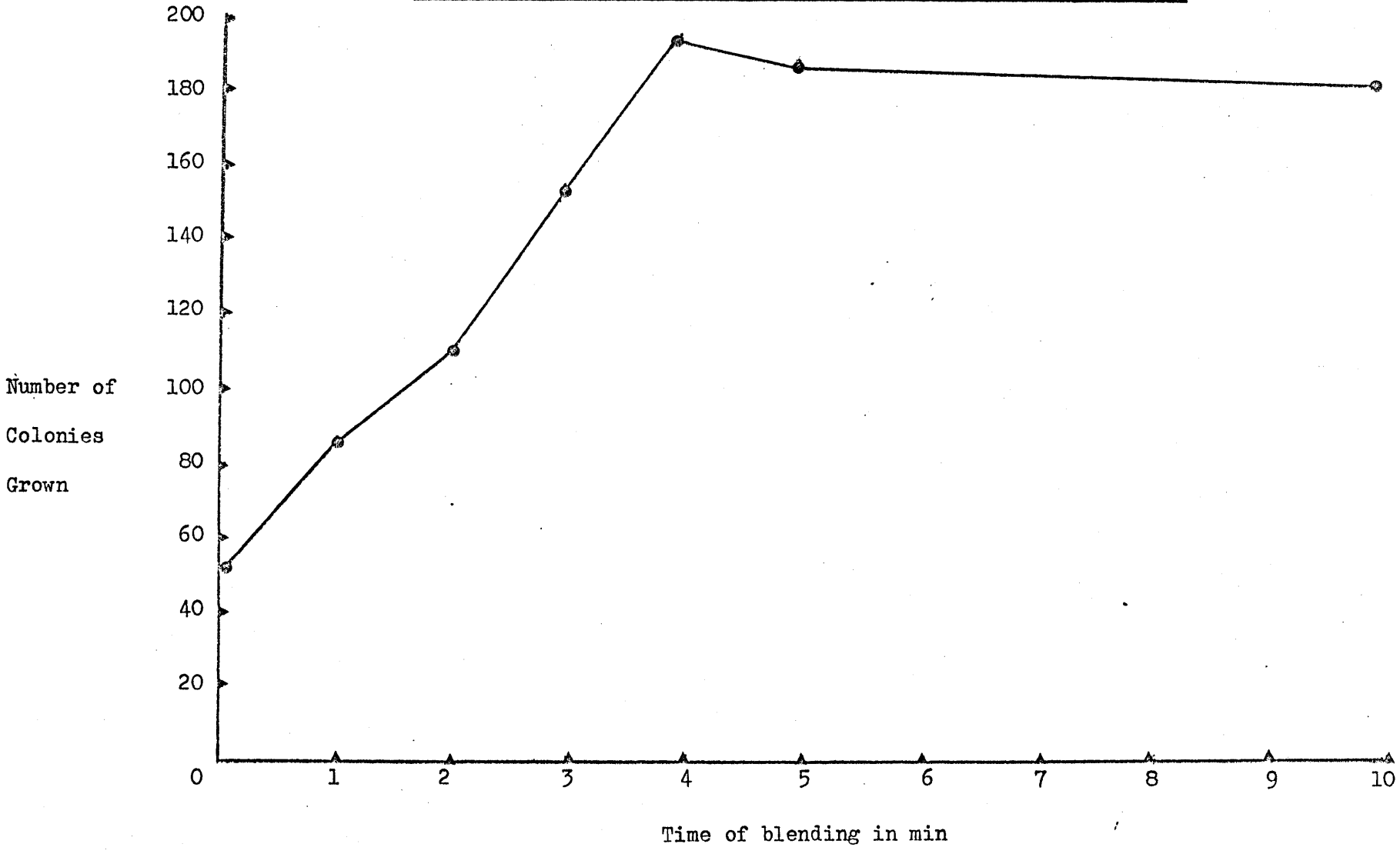
Use of distilled water as diluent (Meadows, 1964).

As addition of detergent, sucrose or glycerol did not give appreciably better counts vigorous mixing was the only technique used throughout the study to increase the number of colonies isolated. Addition of potential nutrients or inhibitors to an isolating medium should be avoided when possible and this was an extra reason for not using additives.

Various diluents were considered. Zobell (1946) advised autoclaved seawater 1 in 3 with distilled water; Kriss (1955) merely recommended a slightly alkaline solution sterilised by filtration; Collins, Jones, Hemlric, Shewan, Wynn-Williams, and Rhodes (1973) suggested 3% sodium chloride solution. The latter had been used in the early trials and it was decided to continue it's use. It was sterilised by autoclaving at 121°C for 15 min.

An Atomix laboratory blender was used to obtain the vigorous mixing of sand and diluent and following initial good results a check on the technique was made to find the optimum time for mixing. Tests were carried out by blending suspensions of sand in 3% sodium chloride solution for 1, 2, 3, 4, 5 and 10 minutes and carrying out viable counts on the supernatants obtained. The mean counts from five plates inoculated after each time-interval were plotted as a graph (Fig. 5).

Fig. 5 NUMBER OF COLONIES GROWN FROM SUPERNATANT AFTER HOMOGENIZATION OF SAND IN 3% SODIUM CHLORIDE SOLUTION FOR INCREASING PERIODS OF TIME



This result is supported by the recently published work of Lee and Calcott (1976). In a Waring blender, optimal blending time was 4 min. Lee and Calcott estimated that they were able to recover 92% of the potential colony forming units present in suspensions treated in this way - the remaining 8% may have been dead at the start or damaged by the blending process. Dale (1974) also used homogenisation to separate bacteria from sand but used a time of 5 min.

Determination of the most appropriate technique for releasing bacteria led to a study of adsorption of bacteria to surfaces.

(2) Cultural conditions

No single temperature is likely to be suitable for the growth of all bacteria present in sand. It seems foolish to give an optimum growth temperature for marine bacteria as was done by both ZoBell (1946) and Kriss (1963), for the minimum growth temperature for some is as low as -7°C and the maximum growth temperature for others is as high as 45°C. However, it is customary to maintain cultures at a stable temperature that will yield maximum numbers of the maximum number of species and most workers isolating micro-organisms from marine environments have chosen a temperature between 16 and 24°C. When trying to cultivate only those organisms active in a substrate it would seem logical to maintain the temperature at a value appropriate to the substrate i.e. in this case, the beach temperature.

It was therefore decided to 'incubate' all cultures at external ambient temperature by using an unheated outside storeroom as incubator. In this building, the temperature varied with that outside except that, as sunlight was excluded, the temperature was shade temperature. A maximum and minimum thermometer was used to record the range to which the cultures were subjected.

The choice of a varying temperature complicated the decision about the length of incubation period. It was decided not to attempt to relate

the incubation period to the temperature. Instead, a standard fixed period of two weeks was used for bacteria. This was, at first, extended to six weeks for fungi but it was found that, with this length of time, fast-growing colonies overgrow the more slowly developing ones and new colonies developed from spores shed by the primary growth. Four weeks was therefore used as standard for fungi.

Consideration had to be given to whether to attempt to isolate anaerobic bacteria. If the top layers of sand could be considered to be well-aerated it was probable that anaerobes were not active there. Webb (1958) has shown that blackening of the sand is a reliable indicator of anaerobic conditions and the black layer in the beaches being sampled was much deeper than the 0 - 1 cm horizon. It was therefore decided to restrict the examination to aerobes.

(3) Media

The sand could be expected to contain a mixture of heterotrophic and autotrophic (including photosynthetic) bacteria. To attempt to isolate all of these would have required a large range of different media making the investigation impossibly cumbersome. It was impossible to state which group of organisms was most 'important'. Since photosynthetic bacteria are difficult to isolate and since there is no single medium which yields a wide range of other autotrophs it was decided to concentrate upon the heterotrophs which, in any case, were more likely to be inter-related with the fungi.

A number of media have been used to cultivate marine heterotrophs many of them being based upon 'aged' seawater or a seawater substitute. The best known of the latter is a formula devised by Zobell (1941) which has been used by many workers with good results. This medium is produced by Difco Laboratories as Bacto Marine Agar 2216 and since use of a single batch of a dehydrated medium, such as this, is of great help in obtaining consistent results it was decided to use this product.

To grow and isolate fungi successfully it is usually considered necessary to remove, or inhibit the growth of bacteria also present in the sample. There are a number of methods of doing this. Modifying the pH of the culture medium to a level unacceptable to many bacteria (pH 5.0 - 5.5) can be used but this means that the pH is unlike that of the original beach substrate. Inhibitory agents may be added to the medium. Brown (1957) when cultivating fungi from sand used the dye Rose Bengal for this purpose. However, such dyes may have a narrow range of anti-bacterial activity and their anti-bacterial effect may vary from batch to batch and must be checked by titration.

Antibiotics are now commonly employed and may be added to the medium; smeared on its surface or be added to the inoculum.

Conant, Smith, Baker and Calloway (1971) recommended chloramphenicol for the isolation of medically-important fungi and used a concentration of 50 mg/l. Seshadri and Sieburth (1971), when culturing yeasts from seaweed, tested penicillin G, streptomycin sulphate, chlortetracycline and chloramphenicol both singly and in combination. They too recommended the use of chloramphenicol but at a strength of 100 mg/l. Roll (1976) had found this level insufficient and quoted a personal communication from Buck who reported that levels up to 300/400 mg/l were necessary to inhibit aquatic bacteria; concentrations of up to 1000 mg/l had not been observed to have any mycostatic effect.

It was decided to add chloramphenicol to the inoculum for this work and so a level of 200 mg/l was chosen as likely to achieve complete inhibition when used in this way.

Media were chosen which would have a similar nutrient composition to the sand so that the results would represent the fungal activity on the beaches. The nutrients available to fungi on the surface of the intertidal zone are mainly derived from organic material left there by the receding tide. This includes litter from a variety of sources and

would include elements from the sewage. Except for the strandline, which was excluded from my sampling area, this litter is almost always in the form of small particles. In addition, dissolved material may be present in the film of water surrounding grains, or, possibly, precipitated upon their surface.

Consequently three media were used:-

Seawater agar - made from aged seawater, collected from the appropriate beach, and filtered through Whatman No. 1 filter paper immediately before use. 2% agar-agar powder was added. After ascertaining that the agar-agar did not alter the pH, no attempt was made to adjust it.

Seaweed agar - this contained 100 g of finely-minced, fresh Fucus in each litre of aged, unfiltered seawater. 2% agar-agar powder was added and again no attempt was made to adjust pH.

Corn Meal agar (Oxoid, CM 103) - this was selected as a standard, simple medium. No attempt was made to increase its salinity.

Fungal isolates were maintained on slopes of malt extract agar (Oxoid CM 59).

It was also decided to try to isolate human faecal bacteria from both beaches. McConkey's agar (Oxoid CM 7) was used. Incubation for this purpose was for 48 h at 37°C.

The samples of faeces were cultured on desoxycholate citrate agar and on 10% horseblood nutrient agar and were also incubated at 37°C for 48 h.

All media were sterilised by autoclaving at 121°C for 15 min.

(4) Viable counts

Each beach was sampled on three occasions for counting purposes

with 5 samples being collected on each occasion. On the first occasion 10 plates of each medium were inoculated from each sample. For subsequent samplings this number was reduced to 7. By trial it was found that a 1 drop inoculum (30 drops = 1 cm^3) of a 1 in 667 dilution of the sand, gave bacterial plates which could be counted quickly and accurately and allowed the colony count to be multiplied by the convenient figure of 2×10^4 to obtain the number grown from 1 g of sand.

For fungal counts a similar 1 drop inoculum was used but the dilution of sand was 1 in 33 giving a factor of $\times 10^3$ for calculating the number of colonies obtained from 1 g of sand.

With all batches of cultures an uninoculated plate was also incubated to check for airborne contamination.

A choice had to be made between the classical 'pour-plate' and 'spread-plate' methods. A number of authors (e.g. Buck and Cleverdon, 1960) have demonstrated that the spread-plate method yields the highest counts of marine bacteria. This is to be expected since for the pour-plate the test material has to be added to the agar while it is still molten, at a temperature of 42°C, and this approaches or exceeds the thermal death point of many marine organisms. This method, however, does give more discrete colonies and discourages spreading organisms. After some experiments, it was decided that the spread-plate was most suitable and in practice there were few problems with spreading organisms. Inoculation was carried out with a standardised dropping pipette and glass spreaders.

(5) Identification

Bacteria

For a single-handed worker with relatively limited resources the problems faced in identifying a large number of isolates of marine bacteria were daunting. It was decided that the only practicable way of tackling the task was to severely limit the number of isolates to be

identified; to use methods which would give maximum information from minimum expenditure of resources and time, and to limit identification, in most cases, to generic level.

Colonies for identification were obtained from the viable count plates. A numbered grid was placed behind the plate and using a random numbers table a square or squares were selected. The colony nearest to the geometric centre of the square was examined, through a hand lens, and its description recorded. Special note of pigmentation was made at this stage since secondary cultures of bacteria often show loss or change of pigmentation (Brown, 1965). Any effects upon the surrounding culture medium were also noted. Colonies were then picked off, sub-cultured onto Zobell's agar and incubated at room temperature (24 - 26°C). Good growth generally occurred within one week.

Each subculture was examined carefully, under the hand lens, for uniformity as judged by the absence of morphologically differing colonies. A single colony was picked off; a smear was prepared in 3% sodium chloride solution, fixed by heat and stained by Kopeloff and Beerman's modification of Gram's stain in which Ziehl-Neelsen's carbolfuchsin, diluted 1 part to 9 parts of water, was substituted for the recommended basic fuchsin solution. This proved necessary because some bacteria stained very weakly.

When the gram-reaction of an organism was uncertain the stain was repeated using a smear of a Bacillus sp. on the same slide as control. Staphylococci are usually recommended as a control of decolourisation but Bacillus is more easily decolourised and thus provides a better control.

Motility was checked by preparing a dilute suspension of the bacterium in 3% sodium chloride and examining this microscopically in a hanging-drop preparation.

All isolates were checked for their ability to grow anaerobically by subculturing onto two Zobell's agar plates, incubating one as a con-

trol and the other anaerobically using the Becton Dickinson Gaspak system.

Gram negative bacilli were then inoculated into 20 test substrates contained within a set of wells in a specially moulded plastic strip.

This is a commercially produced identification system manufactured by API Laboratory Products Ltd. The methodology for the tests is described in detail in the 'profile recognition register' supplied by the firm.

Briefly, the system consists of a range of reagents dried into wells to which a suspension of the bacteria under test is added. Where necessary the well is sealed with sterile mineral oil. The strip is then placed in a sealable plastic tray containing a small amount of water, which acts as a moist chamber. After incubation results are read by noting colour change of indicators, break-up of gelatinised charcoal, or by the reaction to reagents added after incubation (see photograph - Fig. 6).

The test results are converted to a set of figures, which are described as a numerical profile. This profile may be equated with an identity or sometimes alternative identities. When profiles occur which are not identifiable the firm provides a 'phone-in' service and will process data on the unknown through a computer in an attempt to produce an identity. The system is based, of course, on Adansonian numerical taxonomy.

The particular system used was the 'A.P.I. 20E' which is designed for the identification of enterobacteria but which it was hoped would provide sufficient information for the identification of many gram negative marine bacteria to at least genus level.

Advice from the firm's research department was that when using the system for bacteria of non-mammalian origin incubation should be at room temperature for 48 h. This advice was followed.

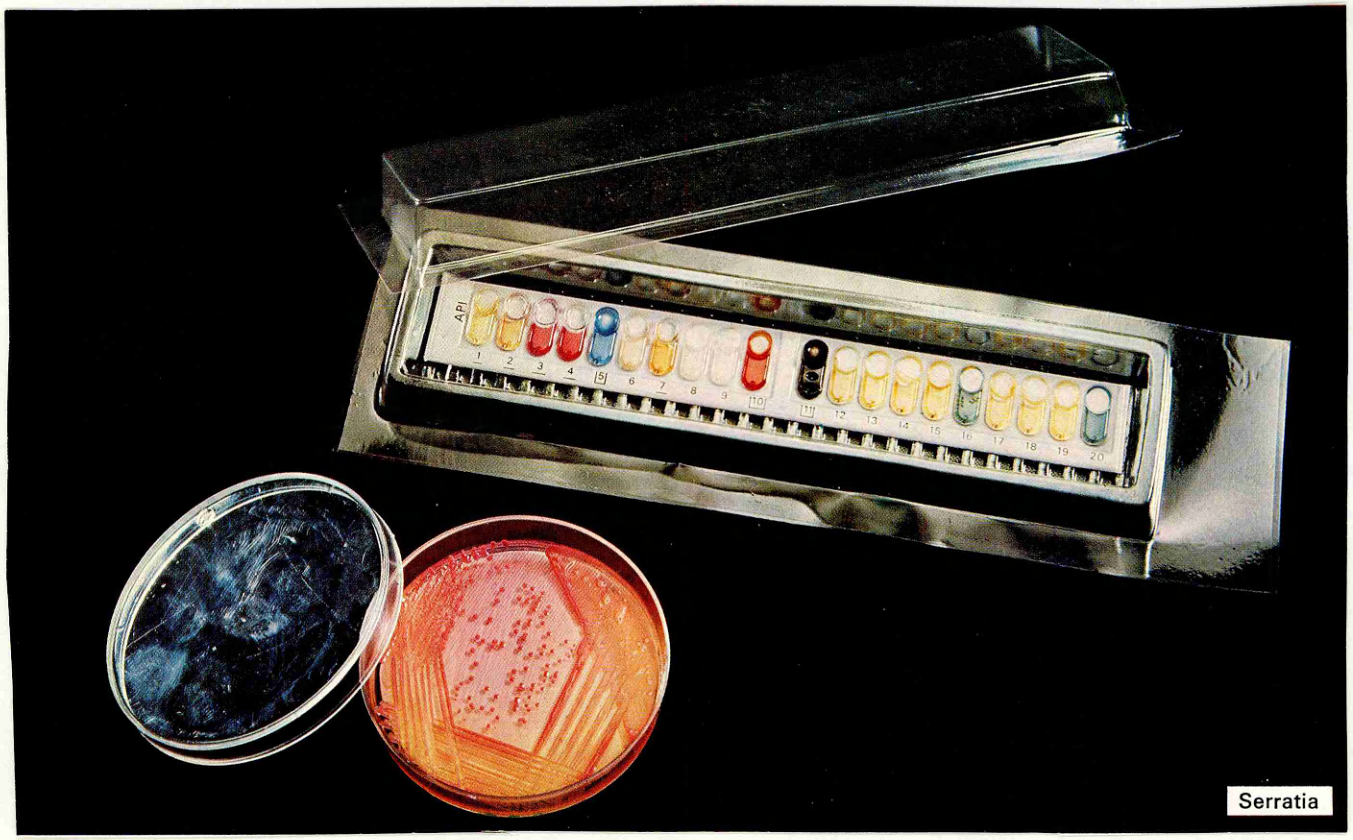
The suspension with which the system is inoculated is normally prepared in sterile distilled water. For oxidase-positive organisms, how-

Fig. 6.

A.P.I. System for testing biochemical activities of bacteria

in order to make identification

Illustrated are the test strips showing the reactions given by Serratia; the container with lid, which provides a moist chamber and a subculture of the bacterium under test.



ever sterile, physiological saline is recommended and has no effect on test reagents. For these investigations physiological saline was used to suspend all bacteria.

The tests carried out by these means were:-

Production of beta-galactosidase (ONPG)

" " arginine dihydrolase

" " lysine decarboxylase

" " ornithine decarboxylase

Citrate assimilation

Hydrogen sulphide production

Urease production

Tryptophane deamination

Indole production

Test for acetoin (VP)

Gelatin liquefaction

Tests for fermentation of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdaline, arabinose.

To these were added tests for oxidase production and nitrate reduction.

Fungi

The identification of fungi is heavily dependent upon examination of reproductive morphology. Where 'sterile' colonies were isolated they were subcultured to various media and incubated at room temperature in an attempt to induce spore formation.

On the first batch of counts from each beach every colony was examined and identification attempted. On subsequent counts only the most frequently occurring colonies were examined and identified, an approach suggested by Dickinson and Kent (1972).

v. STUDIES ON ADSORPTION

The mechanism by which bacteria are adsorbed to the sand grains and the circumstances under which they are adsorbed or released would seem to be factors of prime importance when studying the microbial ecology of beaches. The following experiments were carried out to examine these factors:-

a) Exactly equal weights of sand from freshly collected samples were packed into plastic syringes with a cotton gauze filter, as previously described. A 10 cm^3 volume of freshly collected seawater was run through each column to settle the sand. The weight of sand chosen was such that the settled columns were 10 cm long.

8 x 10 cm^3 volumes of sterile seawater were then run through one column and 8 x 10 cm^3 volumes of sterile deionised water through the other. The last three aliquots were collected and, after dilution, counts of bacteria and fungi in each were carried out. 10 samples were tested in this way.

b) Exactly equal weights of sand, sterilised by gamma radiation, were prepared in 10 x 10 cm columns as described above but in this case using sterile seawater to settle the columns. 10 cm^3 of freshly collected seawater were then run through each column. Counts of bacteria were carried out in duplicate on each aliquot and on the original seawater, after dilution.

c) A dilute suspension of the following organisms were prepared in sterile seawater:-

Pseudomonas (two different species, A & B)

Bacillus

Corynebacterium

Micrococcus

10 cm^3 of these suspensions were run through 10 cm columns of gamma-irradiated sand prepared as above. Duplicate counts of the suspensions were carried out before and after passing through the column.

d) 10 aliquots of freshly - collected sand were placed in sterile, glass, screw-capped bottles and were mixed with 5 cm^3 of sterile seawater on a rotary mixer, at 15 r.p.m. for 10 min. Duplicate counts of bacteria were carried out on the samples from the supernatants.

The same mixtures were then shaken vigorously for 10 min and again duplicate counts were carried out on the supernatants.

The mixtures were then allowed to stand at external temperature for 2 h and the counts were repeated.

The vigorous mixing was repeated, followed by counts, and the gentle mixing was also repeated, followed by counts.

e) 5 x 6 aliquots of freshly collected sand were each washed in 10 x 100 cm^3 volumes of sterile seawater and then bacterial and fungal counts were carried out on the washed aliquots of sand after blending (see p. 26).

At the same time counts were carried out on 5 x 6 g volumes of unwashed sand from the same sample.

vi. STUDIES ON DESICCATION

When surface sand is uncovered by the receding tide organisms may be exposed to progressive loss of water due to drainage and evaporation. This, presumably, would be accompanied by a progressive increase in sal-

inity which theoretically could reach a point where there is no water left and the salts are present in crystalline form. It is important to know to what extent the organisms could survive this process.

To test this, exactly equal weights of freshly collected wet sand were placed on a series of watch glasses which were then placed into sterile petri dishes which had been half-filled with coarse, self-indicating granules of silica gel (B.D.H.). The dishes were carefully sealed with sellotape and sets of desiccant dishes were stored at three temperatures viz:- 4 - 6°C, 24 - 26°C, 36 - 38°C.

At intervals a petri dish from each set was opened and the viability of the bacteria was tested.

This was done by sprinkling a few grains of sand on the surface of a Zobell's agar plate and at the same time viable counts were carried out using the standard method.

vii. STATISTICAL ANALYSIS

Statistical analysis of the data obtained from viable counts was carried out, with the aid of a computer, by Mr. R.A. Reese, B.A., M.Sc. of the University of Sheffield Computing Services. The bacterial and fungal data were compared by site, date and sample using parametric and non-parametric tests.

Correlation of the bacterial and fungal data was also attempted, using the Spearman rank-correlation coefficient test.

F. RESULTS

i. PHYSICAL ANALYSIS

a. Water Retaining Capacity

Tests of water retaining capacity are usually expressed simply as volume of water retained by a unit of sand; often measured by weighing before and after drying. To do this disguises the fact that the ability of sand to retain water is governed by a complex of factors and is not simply a matter of pore space. The sand grains themselves have hygroscopic properties and any organic particles present may be able to absorb water.

Table 1 shows the results of weight loss measurements and the results of water saturation tests. These indicate that the sediments from the two beaches could retain 30 - 33% of their own weight of water. However, if it is accepted that the passage of 5 ml of air, under pressure, was sufficient to empty the capillary spaces then it can be seen that only about one third of this water was interstitial; two thirds was retained by the sediment particles, presumably as surface film, and/or held in the cracks and fissures in the particles' surfaces.

b. Flow Rate

The mean flow-rate through Crimdon surface sand was 59 s for seawater and 60 s for deionised water and at Newburn the means were 74 s and 75 s respectively (Table 2). The flow rate at Newburn was, therefore, 25% slower than that at Crimdon. This was probably due to the higher proportion of fine sand (see grain-size results) and possibly also to the higher proportion of organic particles (see morphology of grains) in the Newburn sand.

Table 1. Water retained (in cm^3) by sediments

(Mean of results from 10 samples from each site. Bracketed figures are results converted to vol. water/vol. wet sand, 100g dry sand = approx. 68 cm^3)

	Newburn	Crinden
Water added to saturate 100 g. dry sand.	30.4 (30.9)	28.9 (29.8)
Water removable by air pressure.	12.8	9.2
Remaining Water.	17.6	19.7
Weight-loss measurements of water contained in freshly collected wet sand.	33.2 (32.8)	33.2 (32.8)

Table 2. Flow-through time in sec of successive additions of 8 x 10 ml seawater and 8 x 10 ml deionized water through sand columns containing 25 g of sediment

Crimdon Sediment

Column	1	2		3	4
Seawater	38	42	Deionized	48	43
	57	61	Water	63	59
	58	59		62	63
	57	59		61	62
	58	58		62	61
	59	57		60	61
	57	57		61	62
	58	58		62	61
Deionized	59	60	Seawater	62	63
Water	60	61		62	62
	58	59		61	60
	59	58		63	61
	58	59		62	62
	60	57		61	61
	58	58		61	61
	59	58		60	61

Newburn Sediment

Column	1	2		3	4
Seawater	49	53	Deionized	58	50
	67	74	Water	85	68
	66	74		82	70
	66	77		82	71
	65	77		83	70
	65	76		81	69
	66	76		82	71
	65	77		83	70
Deionized	65	79	Seawater	79	72
Water	67	77		80	71
	66	78		82	70
	67	79		81	71
	67	78		82	72
	67	78		80	70
	68	78		81	71
	67	79		81	71

There was no significant difference between the flow-rates of seawater and deionised water. This finding seems to conflict directly with that of Anderson and Meadows (1969) who noted that the flow-rate of deionized water decreased by about 50% and that of seawater by only 10% when run through long columns of washed sand which had been plugged with glass wool. Glass wool was tried as a plugging device in this work but was found to interfere with flow-rate in an inconsistent manner and it is thus possible that this factor affected Anderson and Meadows' results.

c. Water Content

The tests of water content were carried out on a day when ambient air temperatures were 22 - 24°C. In these conditions the mean water content of surface sand from five sampling points in the intertidal zone of Newburn beach was 33.8 vols. water/vol. wet sand, immediately after emersion. This figure fell steadily during the period the sand was exposed to a level of 17.4 vols. water/vol. wet sand. Just prior to the incoming tide covering the sampling points the figure rose sharply to the point just below its original level (Fig. 7).

The corresponding results from Crimdon beach were almost identical with the immediate post-emersion figures giving a mean of 33.2 vols. water/vol. wet sand reducing to 18.6 vols. water during the emersion period.

d. Grain Size

The median particle size was almost the same for each site (Crimdon 454 μm; Newburn 450 μm) and both beaches have a majority of particles in the 425 - 500 μm (i.e. medium) class (Table 3.). However, as the accompanying histograms show (Fig. 8.), the two sediments are otherwise quite different.

Fig. 7 Water content (vols. water/100 vols. wet sand) of surface sand at both sites after increasing periods of emersion

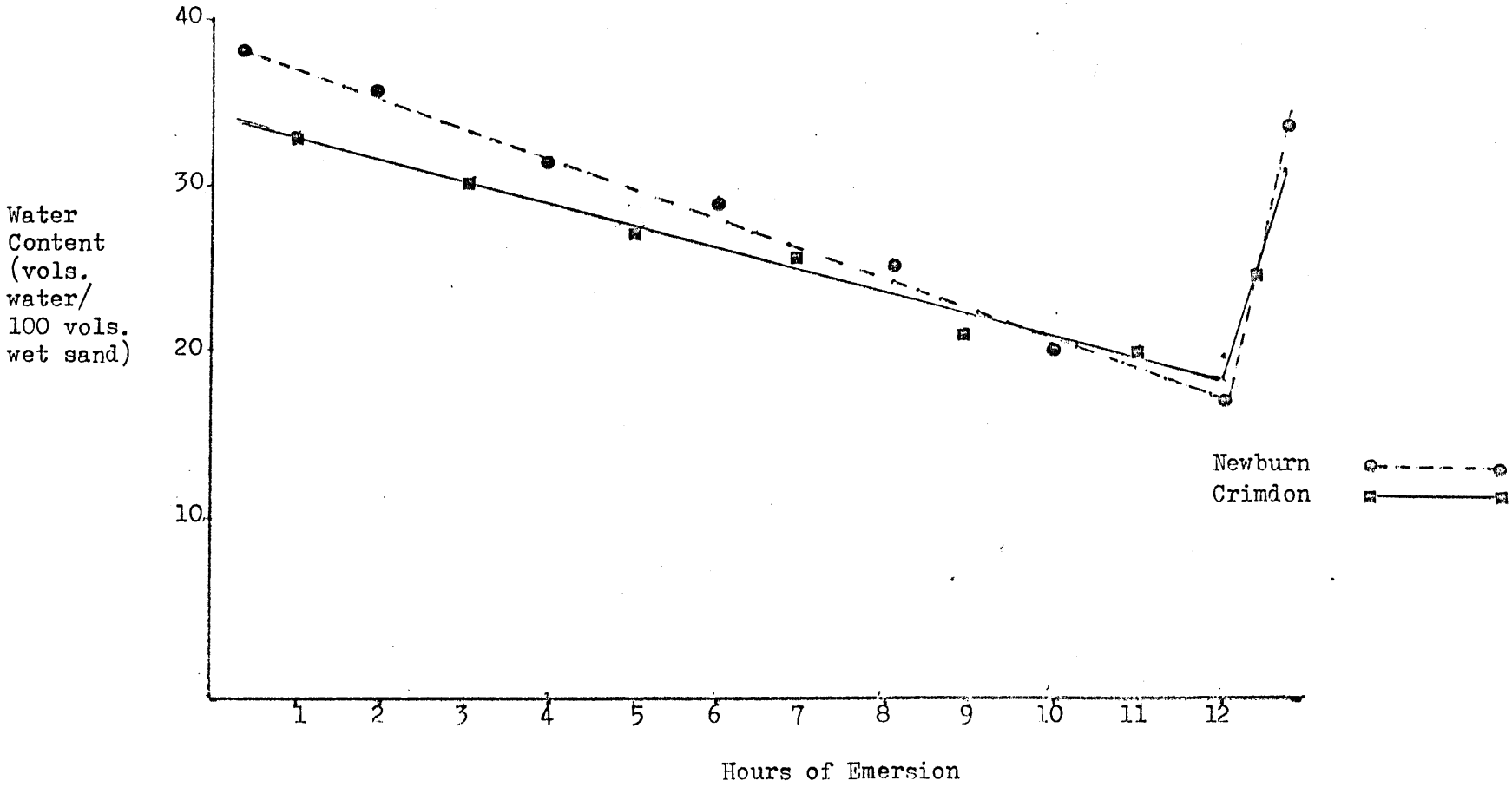


Table 3. Analysis of particle size of four aliquots of sediment bulked from 10 samples

	Size range (μm)	Weight (g)		Size range (μm)	Weight (g)
Sample 1	<300	36.58	Sample 2	<300	33.97
	300 - 355	0.68		300 - 355	2.62
	355 - 425	2.35		355 - 425	1.49
	425 - 500	70.02		425 - 500	78.12
	500 - 730	8.61		500 - 730	5.03
	>730	14.43		>730	6.99
Sample 3	<300	32.75	Sample 4	<300	45.62
	300 - 355	1.24		300 - 355	2.40
	355 - 425	1.34		355 - 425	1.19
	425 - 500	47.07		425 - 500	40.79
	500 - 730	8.94		500 - 730	6.89
	>730	12.27		>730	11.57

Means	Size range (μm)	Weight (g)	%
	<300	37.23	31.49
	300 - 355	1.73	1.46
	355 - 425	1.59	1.34
	425 - 500	59.00	49.90
	500 - 730	7.37	6.23
	>730	11.31	9.57

Median particle size = 450 μm

Table 3 (cont.)

CRIMDON

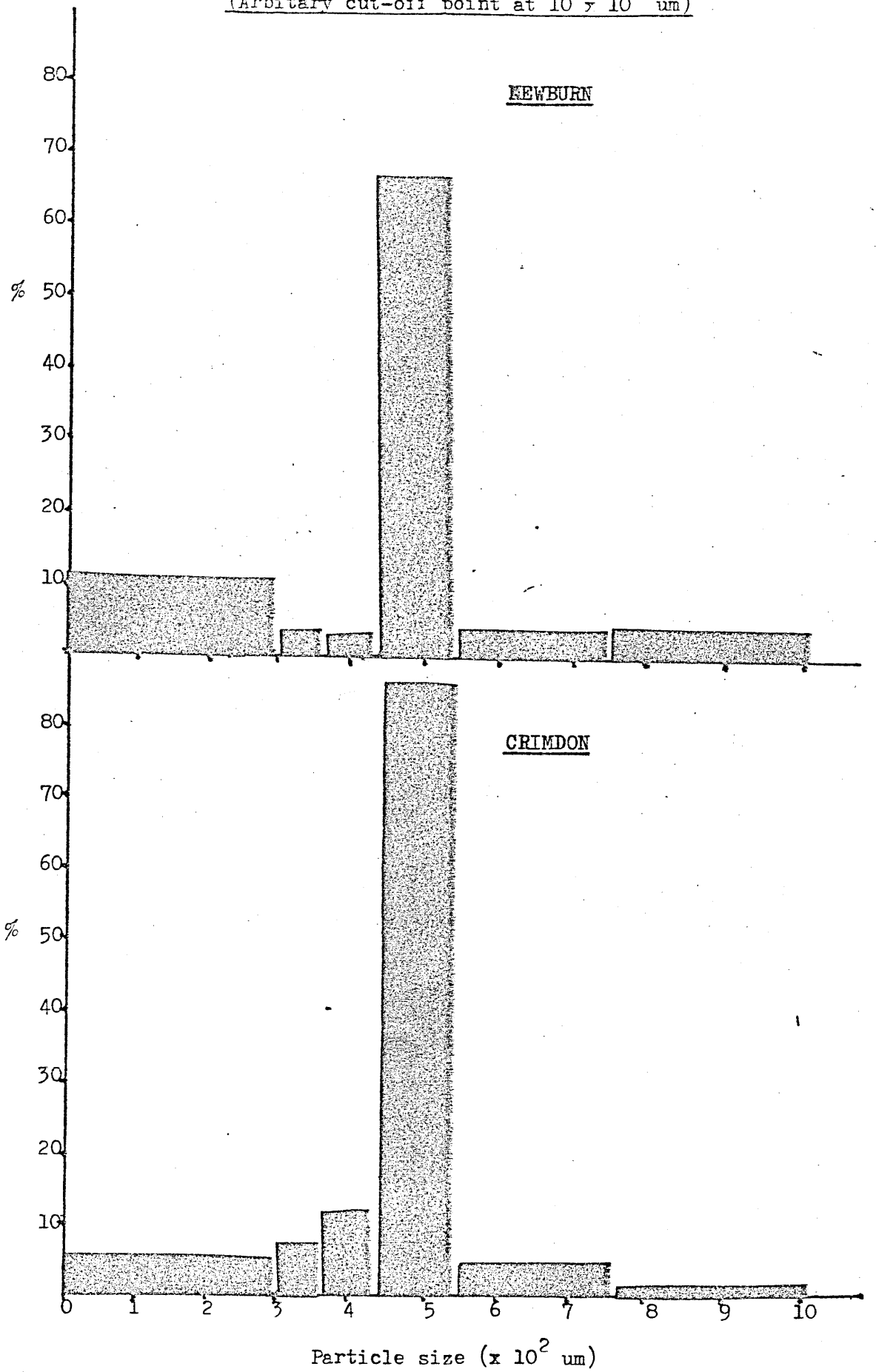
	Size range (μm)	Weight (g)		Size range (μm)	Weight (g)
Sample 1	< 300	16.33	Sample 2	< 300	17.7
	300 - 355	6.52		300 - 355	3.02
	355 - 425	4.78		355 - 425	12.13
	425 - 500	77.07		425 - 500	85.81
	500 - 730	12.33		500 - 730	10.66
	>730	0.32		>730	0.12
Sample 3	< 300	16.08	Sample 4	<300	25.52
	300 - 355	4.23		300 - 355	7.66
	355 - 425	16.17		355 - 425	8.84
	425 - 500	79.02		425 - 500	97.46
	500 - 730	19.37		500 - 730	14.29
	>730	0.29		>730	0.19

Means	Size range (μm)	Weight (g)	%
	< 300	18.91	14.11
	300 - 355	5.36	4.00
	355 - 425	10.48	7.82
	425 - 500	84.84	63.32
	500 - 730	14.16	10.57
	>730	0.23	0.17

Median particle size = 450 μm

Fig. 8 Particle size distribution at the two sites

(Arbitary cut-off point at 10 x 10^2 um)



The sediment from Newburn shows a high level of fine particles which is counterbalanced by an admixture of medium to coarse sand. (The words coarse, medium and fine are used in accordance with the definitions of Ritchie and Mather, 1969). This result fits with the known conditions on the beach i.e. a fine sandy beach from which the finer grains are being eroded and replaced by coarser particles. Crimdon's sediment shows more evenly sorted particles with a significantly smaller proportion of coarse grains. It is probable that Crimdon was a beach of medium sand on which fine sand is now being deposited.

c. Morphology of Grains

The sediments were almost identical in content except for a significant difference in the percentage of 'other organic' particles (Table 4). It is assumed that the 'cellular organic fragments' were seaweed remnants and that the other organic material originated mainly from sewage and 'stormwater'. This would account for the higher proportion of these particles present at Newburn.

The content of organic fragments on both beaches was higher than anticipated and it is clear that, as these particles represent 8 - 13% of the whole they could represent a significant ecological factor. The coal particles and sea shell fragments present were not angular or creviced but were smooth rounded particles.

The siliceous grains were angular, irregularly shaped, refractile particles, almost all of which showed numerous cracks and fissures. Many showed heavy deposits of a fine, reddish-brown deposit both on the surface and within the particles. The addition of 2,2[1] dipyridyl reagent caused immediate colour change of these surface deposits. As this reagent is specific for iron this identified them as iron salts - probably ferrous oxides (see Pettyjohn, Potter and Siever, 1973). Meadows and Anderson (1968) have described a stainable deposit on the

Table 4. Counts of different types of particle: means from 10 samples each of 500 particles, expressed as a percentage

	Crimdon	Newburn
	%	%
Siliceous particles	85	80
Sea-coal particles	6	5
Shell fragments	1	2
Cellular organic fragments	1	1
Other organic fragments	7	12

surface of sand grains and suggested that this could be an organic substance, possibly extracellular products of bacteria. Another possibility would be that it was some sort of 'humic' deposit. Attempts to dissolve the deposit in graded strengths of alkali at temperatures up to 60°C were unsuccessful, suggesting it was not humus. Similar tests with acid solutions also failed to dissolve the material but did cause vigorous production of gas, presumably carbon dioxide, from shell fragments. Occasionally, particles which were apparently organic also showed scanty gas production indicating the presence of calcium carbonate deposits within the particle.

Floodgate (1965) has suggested that bacteria may precipitate calcium carbonate in situ from seawater by a local pH change, and it is possible these deposits were produced in this way. It is equally possible, however, that these particles were decaying animal remains containing skeletal fragments.

It is thus not clear whether the reddish-brown deposits seen here were the same as those seen by Meadows and Anderson (1968) but careful examination did not reveal any iron-negative precipitates except where the deposit was embedded within the grain and, therefore, inaccessible to the reagent.

ii. CHEMICAL ANALYSIS

a. Salinity

The sodium and chloride levels in the sand were higher at Crimdon whilst potassium levels were higher at Newburn (Table 5a). However, variability between samples was three times greater at the Crimdon beach.

Calculation of the salinity by multiplying the mean chlorinity by 1.80655, as recommended by Perkins (1974), gives a result of 3.34% (33.4 g/kg) at Crimdon and 3.59% (35.9 g/kg) at Newburn.

Estimates of bicarbonate (including dissolved CO_2) and of urea (the method is not affected by ammonia) all gave results of <2 mmol/l.

The results obtained in these tests are actually those for 'resting water', as the samples were, of necessity, collected while the surface sand was still visibly wet from the receding tide. It was expected, therefore, that they would not differ greatly from those of seawater. However, as shown in Table 5b the salinity of the surface seawater from Hartlepool Bay was 0.13% less than that at Crimdon beach and 0.38% less than at Newburn. In addition, the beach salinities may have been reduced from an even higher level by capillary rise of fresh-water originating from land drainage and more particularly from the freshwater stream at Crimdon and the storm drain at Newburn.

The inherent variability of the chloride method is approximately 1%. It can, therefore, be stated that there was true variation from sample to sample, at both sites, this being significantly greater at Crimdon.

It was thought possible that the 'bicarbonate' results were low because of CO_2 loss in the settling period of 18 - 20 h. They were, therefore, repeated on resting water pressured out of freshly collected sand samples and tested within one hour without dilution. A level of 2 mmol/l was obtained from 5 samples from each site with no variation.

There was no variation in magnesium levels but the Crimdon levels were slightly lower than those of Newburn.

b. Organic Carbon

The mean content of Newburn sand was 22% greater than that of the Crimdon material (Table 6). Variability as expressed by the co-efficient of variation was 6.6% higher at Crimdon.

Table 5a. Levels (mmol/l) of magnesium, sodium, potassium and chloride present in samples of 'resting water' from surface sand

	CRONDON				NEWBURN			
	Mg	Na	K	Cl	Mg	Na	K	Cl
	50	476	10.0H	548	47.5	2640L	10.0	548
	50	472	10.0	548	47.5	468	10.0	560
	50	424	8.8	492	47.5	480	10.0	568H
	50	468	9.6	540	47.5	468	10.0	558
	50	440	9.2	520	47.5	480H	10.0	568
		468	10.0	552		472	10.0	560
		468	9.6	548		464	9.6	552
		420	8.8	484		464	9.6	556
		468	9.6	544		472	10.0	564
		432	8.8	508		468	10.0	560
		480H	10.0	560H		452	9.6	540
		460	9.6	536		464	9.6	552
		424	8.8	492		468	10.0	556
		460	9.6	540		464	10.0	552
		432	8.8	508		464	10.0	560
		472	10.0	548		464	10.4H	560
		404	8.4	472		468	10.0	568
		392L	8.0	452L		468	10.0	564
		456	9.6	528		468	10.0	560
		436	9.2	508		464	10.0	564
		472	10.0	548		432L	9.2L	524L
		396	8.0L	456				
		464	9.6	540				
		460	9.6	536				
Mean	50	448	9.3	521	47.5	465	9.9	557
S.D.	Nil	34.16	0.686	33.48	Nil	9.86	0.250	10.50
C.V.	Nil	7.30%	7.30%	6.42%	Nil	2.12%	2.53%	1.88%
SALINITY				3.34%				3.59%

(H = Highest level; L = lowest result; OL = result considered as outlier and not included for calculation of Mean; S.D. or C.V.)

Table 5b. Levels (mmol/l) of sodium, potassium and chloride in 5 samples of surface seawater from Hartlepool Bay

	Na	K	Cl
	496	10.8	502
	502	10.8	502
	504	11.0	496
	500	10.6	498
	498	10.8	502
Mean	500	10.8	500

SALINITY 3.21%

Table 6. Organic carbon content (mg/kg) in surface sand; 10 samples collected from the mid-tide line of each beach on the same day

	HEMBURN	CRIMDON
	730	510
	840	610
	1000	630
	980	700
	880	840
	800	800
	880	870
	950	800
	950	750
	900	800
MEAN	889	731
S.D.	83.0	116
C.V.	9.3%	15.9%

The higher levels of organic carbon at Newburn were anticipated because of the larger percentage of organic fragments counted there. However, direct correlation of the percentage of organic fragments in the sand with mean measured organic carbon indicated that the mean of the Newburn results should have been almost 50% greater than that of Crimdon instead of the actual 22%. It was possible, therefore, either that at Crimdon there was a source of carbon, other than the fragments, which narrowed the difference or that a difference in size or specific gravity of the organic particles was sufficient to account for lack of correlation.

A complicating factor was the presence of coal particles at both sites which might have been included in the carbon measured (see Southward, 1952). Samples of drifted sea-coal treated in the same way as the sand samples gave results of less than 2 mg/kg carbon in sea-coal. This low result was presumed to be due to the resistance of the coal particles to acid-digestion. However, the coal particles in drifts are much larger than those found generally distributed in the sand so tests were repeated using drift-coal ground to a fine powder. In this case the method gave a result of 10 000 mg/kg sea-coal; in other words 1% of the sea-coal was being measured as organic carbon by the method.

As found by Southward (1952), there proved to be no way of removing sea-coal from the sand without also removing other organic particles. It's contribution to the mean organic carbon result, therefore, had to be calculated assuming that the percentage of counted particles provided a reasonably accurate guide to the volume of sea-coal present. This calculation gave figures of 0.44 mg (Crimdon) and 0.45 mg (Newburn) as the contribution of sea-coal to the mean estimated organic carbon. These results cannot be considered as accurate but are sufficiently so to indicate that such a contribution can be ignored in considering the overall organic carbon levels.

c. Nitrogen

Analysis of nitrogen in five samples from each site showed that the mean result from Newburn sand was higher than that of Crimdon (Table 7). Although the numbers of samples are too small for statistical analysis the results are grouped closely enough to assume use of a mean is valid and it appears that variation was again probably greater at Crimdon.

Newell (1970) has suggested that organic carbon represents about 50% of the organic matter present in marine deposits, and, that nitrogen represents 7.5%. Using these figures it is possible to derive expected figures for nitrogen from the organic carbon results viz:- Newburn 133 mg/kg and Crimdon 109 mg/kg. If Newell's factors are accepted there appears to be an additional source of nitrogen at both sites, but particularly at Newburn. It is probable that these sources are the sewage outlet at Newburn and a freshwater stream at Crimdon.

The carbon/nitrogen (C/N) ratios calculated from the means are Newburn - 3.7; Crimdon - 5.6; these are much lower than the figures recorded by Longbottom (1968) for sediments from the intertidal zone of the north Kent coast which averaged 9.0. Since organic material of algal or plant origin could be expected to increase the C/N figure it is probable that additional nitrogen was being contributed at both sites in the form of nitrites or nitrates with, perhaps, the addition of ammonia at Newburn.

Table 7. Total nitrogen content (mg/kg) of five samples of air-dried, surface sand from each beach

	Newburn	Crimdon
	260	120
	250	100
	220	160
	230	170
	230	100
Mean	238	130

iii MICROBIOLOGICAL ANALYSES

a. Direct Observation

Examination of the unstained, unfixed sand preparations showed the presence of occasional diatoms and flagellates. Some of the diatoms appeared to be firmly attached to sand particles which fits with the findings of Colocoloff and Colocoloff (1972) who in their studies of deep sand primary production found that they needed to use ultrasonic emissions to separate diatoms from sand.

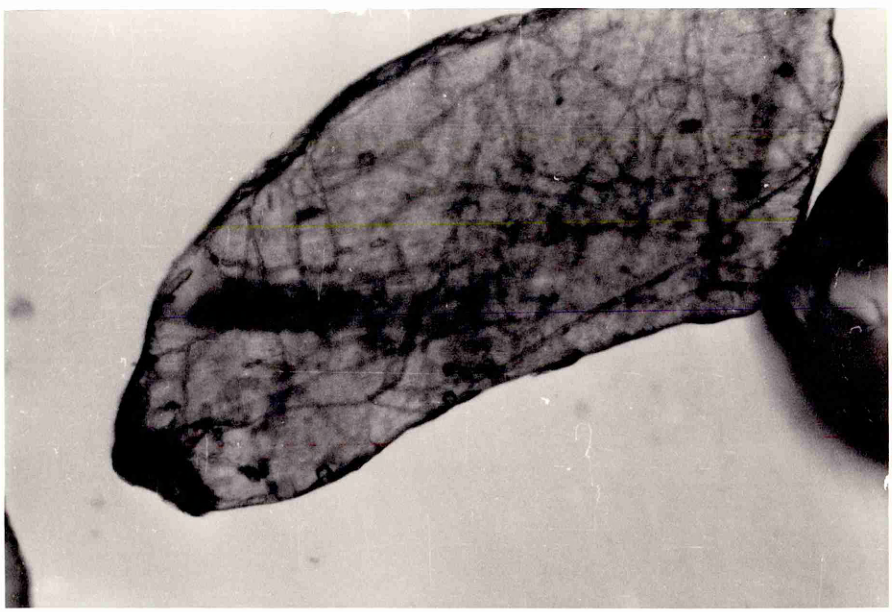
Fungal hyphae could be clearly seen forming a mycelium within many of the organic particles (see photograph, Fig. 9) and also a few of the shell fragments. An occasional siliceous particle also showed the presence of fungal hyphae attached to the surface.

Bacteria were also present; many of them being very short motile bacilli with a quick, darting form of motility. There were also longer rods which showed a slower, steadier movement. The short bacilli were most often seen in the immediate vicinity of the particles.

Stained preparations confirmed the presence of fungal hyphae within some particles and occasional free spores were also seen. These preparations also showed bacteria attached to the surface of the sand grains. When fixed and stained it was often difficult to identify these as bacteria, with certainty, particularly when they were attached to heavily etched or pigmented surfaces. The staining was often very weak and the bacteria very small. This confirms the experience of Rheinheimer (1974) who found that direct counts were very difficult on marine organisms in sediments due to the fact that the bacteria were 'stunted'. On the surface of some particles much larger rod-like organisms, which stained more heavily, were present amongst the short bacilli. It was uncertain whether these were bacteria or blue-green algae.

Fig. 9

Fungal hyphae forming a mycelium within organic particles from beach sand sample



x 100

x 400

Microscopic examination of the agar block cultures, as they grew, showed that in the early stages of growth, bacteria multiplied rapidly at the interface between the particles and the medium and a surface layer of small motile bacilli could be seen to form around each particle. Some of these bacilli showed the strange 'propeller-like' or tumbling motion described by Marshall, Stout and Mitchell (1971). Some larger bacilli, however, behaved differently. They were free in the medium and appeared to make repeated attempts to approach a particle only to be repelled at a certain distance, as if by an unseen barrier. This behaviour was seen often enough for it to be considered more than a chance phenomenon.

Where the agar-block cultures were carried out on chloramphenicol-treated medium the growth of fungal hyphae could be clearly observed and when this mycelium reached the edge of the agar block sporing occurred. It was not always possible to identify the origin of growth and in these cases it is assumed that the growth originated from an unseen spore. However, in some cases growth definitely originated from the hyphae seen within an organic particle proving that such hyphae were viable.

Direct counting by both methods described proved to be technically difficult and time-consuming. With the Jones and Mollison technique, the prepared films were very friable due to the tiny fragments of sand present and they were easily fragmented whilst being removed from the chamber and floated onto the slide. Increasing the concentration of agar was of some help in preventing this. Using the method described by Parkinson, Gray and Williams (1971) there was uneven drying of the films resulting in local concentrations of organisms on particular parts of the slide. As the authors point out, this means that the whole smear must be counted if gross inaccuracies are to be avoided. Again the weak staining and small size of the bacteria often made them difficult to distinguish from the sand detritus.

A series of counts were carried out using the methods of both Jones and Mollison (1948) and Parkinson et al. (1971) and the estimated number of bacteria present varied from $3 \times 10^6 - 10 \times 10^6$ /g of dry sand (Table 8).

These results were very much lower than those obtained by the direct counting methods used by Anderson and Meadows (1969). They obtained counts of from $140 \times 10^6 - 1163 \times 10^6$ /g of dry sand, a range much higher than those reported by other workers.

Attempts to measure the biomass of fungal mycelium were also made but the number of hyphae seen was so small that this was not possible. This accords with the experience of Brown (1958a) who found that mycelium was 'virtually absent' from her Jones and Mollison preparations. She suggested that this was due to loss of coarse particle fractions to which the hyphae had been closely adherent.

In view of the unsatisfactory results obtained by these methods it was decided that they did not provide an accurate means of estimating bacterial or fungal biomass.

b) Quantitative Studies

Bacteria

Sediment samples from the surface sand of Newburn beach gave viable counts which varied from $1.33 \times 10^6 - 7.40 \times 10^6$ /g wet sand, whilst samples from Crimdon gave a range of $0.39 \times 10^6 - 11.87 \times 10^6$ /g wet sand (Table 9a).

There was some indication that the viable count of bacteria was affected by temperature - either the temperature of the sediment at the time of sampling or the subsequent temperature of incubation (Table 9b and Figs. 10 a, b, c and d)

It should be noted that all these cultures were set up from freshly

Table 8. Estimated number of bacteria ($\times 10^6$/ g dry sand) present in 5 samples of Newburn surface sand using two direct counting techniques

Sample	Jones & Mollison method	Parkinson, Gray & Williams method
1	8.2	4.2
2	7.4	5.0
3	4.6	3.84
4	10.0	6.8
5	5.4	4.4

Table 9a. Means of 7 replicates (10 in the case of Newburn - 23.11.75) of bacterial counts ($\times 10^6$/ g wet sand) from 5 samples of sand collected on three occasions from each beach

NEWBURN

Sample	Date:-	23.11.75	31.10.76	7.11.76
1		4.14	2.89	4.80
2		3.64	1.96	7.40
3		4.71	3.40	1.37
4		5.22	2.57	1.33
5		5.66	3.90	1.79
Overall mean		4.67	2.94	3.34

CRIMDON

Sample	Date:-	11.5.76	11.8.76	13.3.77
1		11.87	3.59	3.15
2		7.79	0.54	3.89
3		1.12	0.39	1.64
4		-	0.56	2.97
5		2.02	0.39	3.85
Overall mean		5.70	1.09	3.10

Table 9b. Sand temperatures at time of sampling (S.T.); mean daily temperature of incubation (M.D.T.); temperature extremes to which cultures were subjected (T.E.) and overall mean bacterial counts ($\times 10^6$/ g wet sand)

HILBURN

Sampling date	S.T. (°C)	M.D.T. (°C)	T.E. (°C)	Count
25.11.75	5.9	4.9	0.1 - 9.4	4.67
31.10.76	4.8	4.6	0.9 - 9.6	2.94
7.11.76	4.0	4.5	0.7 - 8.2	3.34

CROMDON

Sampling date				
11.5.76	8.0	12.1	9.4 - 15.5	5.70
11.8.76	14.7	17.3	15.0 - 20.0	1.09
13.3.77	5.5	6.5	2.2 - 9.4	3.10

Fig. 10a. Bacterial Numbers/Sand Temperature at time of sampling

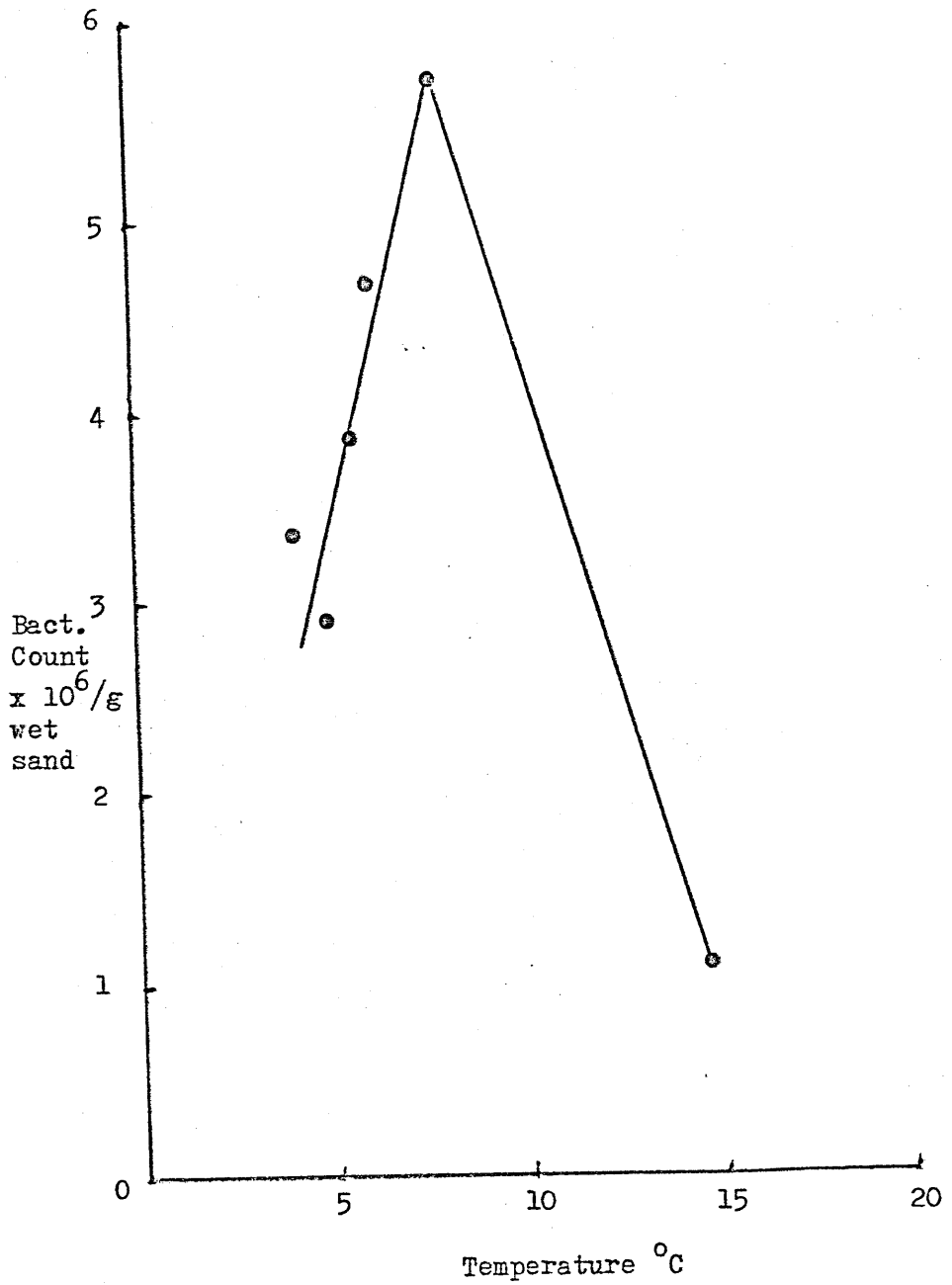


Fig. 10b. Bacterial Numbers/Mean Daily Incubation Temperature

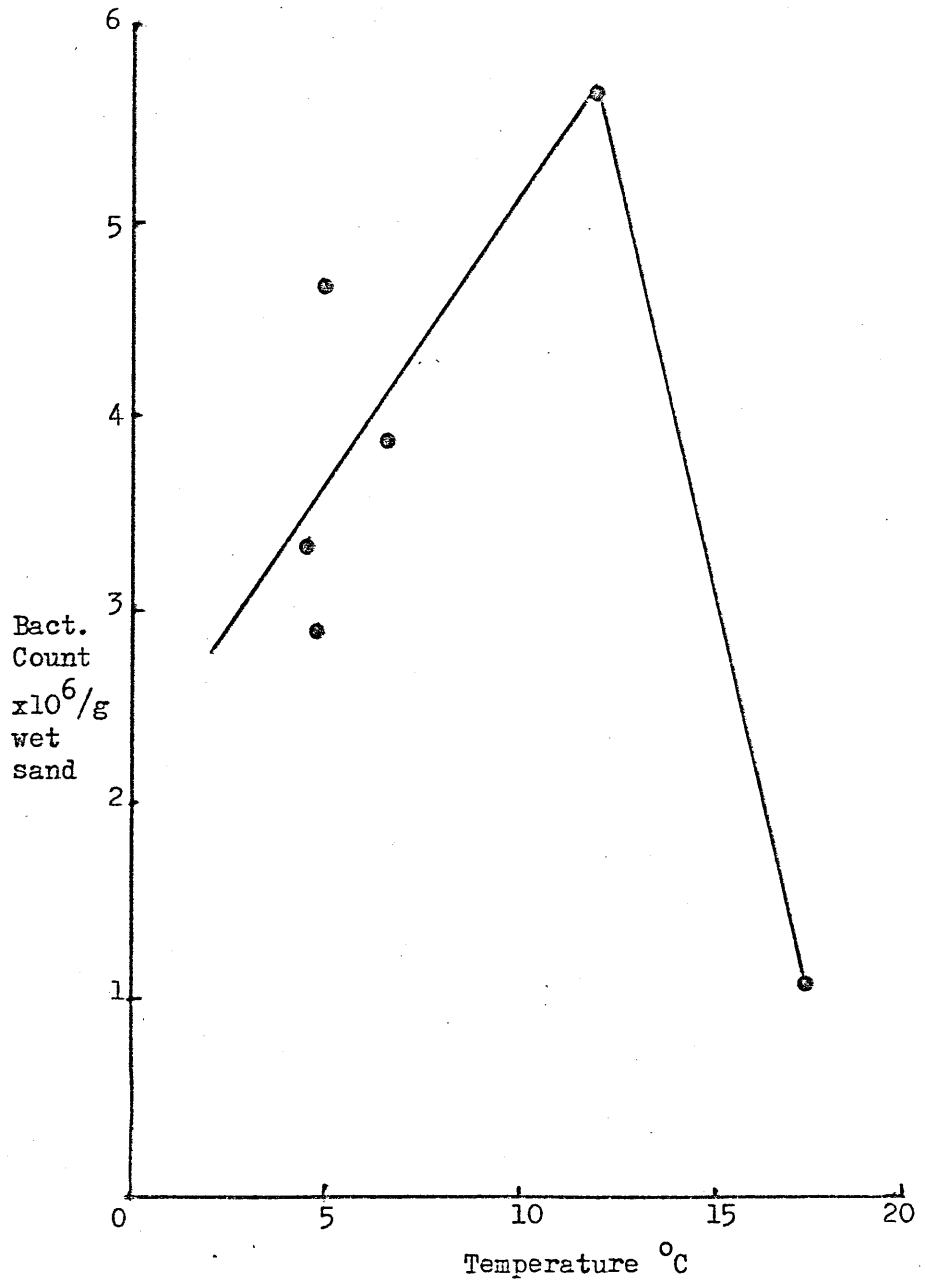


Fig. 10c Bacterial Numbers/Month of Sampling Visit

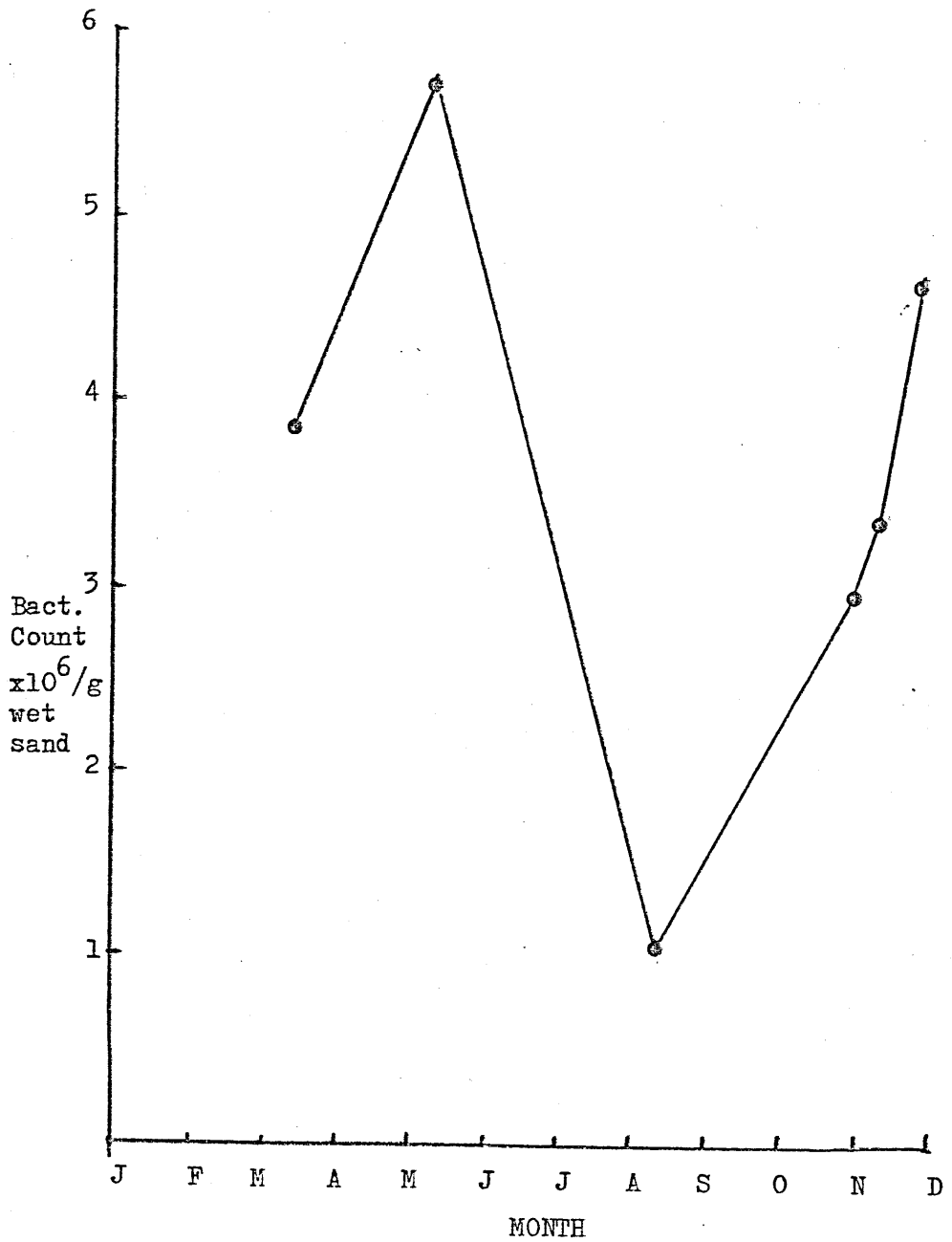
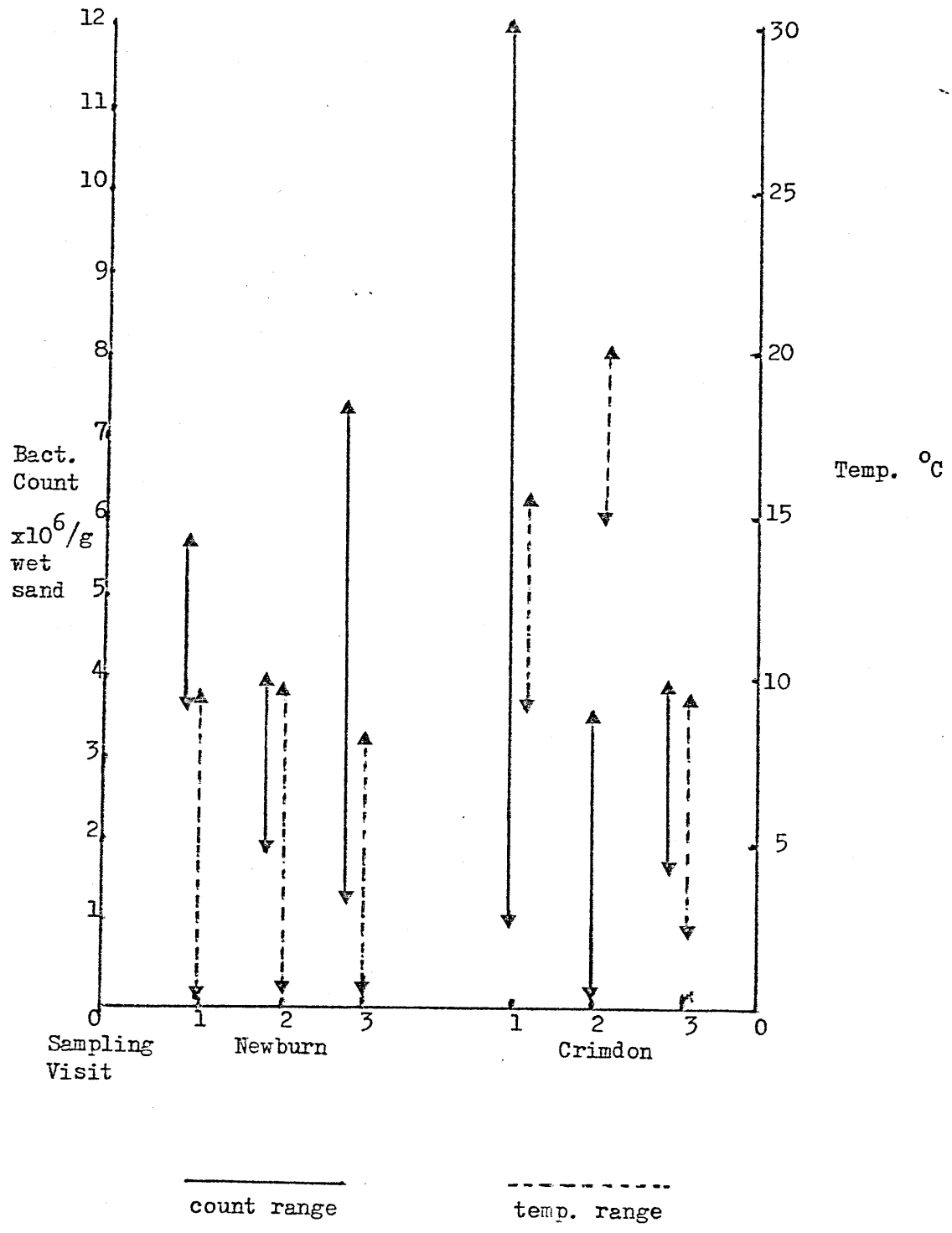


Fig. 10d. Range of Bacterial Counts from six samples/Range of Temperature of Incubation (= Ambient Shade Temperature)



collected wet samples of sand and they, therefore, include organisms from both the surface of the grains and the interstitial water. Counting of only those organisms attached to the grains was considered but it was felt that estimation of total numbers of viable organisms present was more appropriate for this investigation. However, a smaller series of tests was made to estimate the proportion of organisms attached to the grains and these are reported later (Table 19).

It will be seen from the detailed results (Appendix I) that of the 225 plates used for bacterial counts only 3 were 'spoiled' by spreading organisms. Sample 4 from Crimdon on 11.5.76 gave counts which were over the countable figure (i.e. 20×10^6/ g of wet sand).

This was not due to a spreading organism but to very large numbers of tiny colonies and the probable explanation was an unseen pocket of organic matter close to the sampling point.

Fungi

The mean number of colonies on seven plates of each of four different media from samples of Newburn sand varied from 32 - 111; at Crimdon the figures were 89 - 857 (Table 10a). There was no clear relationship between temperature of sediment at time of sampling (and/or incubation temperatures) and the number of fungi cultured although there was some suggestion that high temperatures were associated with higher numbers of fungi (Table 10b). Detailed results for the fungal colony counts are shown in Appendix II.

It should be noted that the total number of colonies given in Table 10a includes those colonies which were grown on the Zobell's medium which was also used to isolate bacteria. The dilution used to inoculate these plates was thus 20 x greater than those used for the fungal plates. Since the figures for fungi isolated are not to be regarded as 'absolute numbers' of fungi present in the sand it was decided

Table 10a. Total numbers of colonies of fungi grown on 7 plates of each of 4 media from 5 samples of wet sand from both beaches collected on three occasions from each site

KINSON

Sample	Date:-	25.11.75	31.10.76	7.11.76
1		81	55	33
2		13	109	45
3		26	209	194
4		14	60	164
5		28	122	101
Mean		32	111	107

CRICHEL

Sample	Date:-	11.5.76	11.8.76	15.3.77
1		56	1949	315
2		31	1483	455
3		31	240	165
4		246	386	216
5		81	226	596
Mean		89	857	349

Table 10b. Sand temperatures at time of sampling (S.T.); mean daily temperature of incubation (M.D.T.); temperature extremes to which cultures subjected (T.E.) and mean number of colonies of fungi

Sampling date	S.T. (°C)	M.D.T. (°C)	T.E. (°C)	Number of Colonies
HEMBURN				
25.11.75	5.9	4.6	0.1 - 9.4	32
31.10.76	4.8	5.6	0.7 - 9.6	111
7.11.76	4.0	4.4	0.0 - 9.8	107
CRIMDON				
11.5.76	8.0	12.5	9.2 -18.4	89
11.8.76	14.7	16.4	11.1 -20.0	857
13.3.77	5.5	3.7	1.7 - 9.4	349

to include those figures as they stood for recording and statistical purposes.

However, to compare the number of fungi grown on the different media it is necessary to multiply the numbers grown on Mobell's by this factor of 20. When this is done it appears that this medium, designed for bacteria and on which bacterial colonies were present yielded significantly higher numbers than the other three (Table 10c).

c. Statistical Analysis

Bacteria

The data presented to the computer can be described as having a hierarchical structure:-

2 sites

3 dates at each site

5 samples on each date

10 or 7 plates for each sample.

The first task was to determine the probable type of distribution of the bacterial data. The most commonly found distribution is the Gaussian or 'normal' distribution, in which each of a set of measurements is an approximation to the same value with a random error term added.

This hypothesis was tested for its validity by applying to each sample the Kolmogorov-Smirnov goodness of fit test. For none of the thirty samples was this hypothesis remotely near rejection. Typical two-tailed probability results varied between 0.9962 and 0.9482. This suggests that the assumption of a normal distribution was acceptable.

For comparison, the same test was carried out using the hypothesis that the distribution was uniform and in this case probabilities were consistently lower and in one case the hypothesis was rejected at the 5% level.

Table 10c. Total number of colonies grown on four different media and mean daily temperature of incubation

(Figures for Zobell's medium are multiplied by 20 to allow for extra dilution)

Sampling date	M.D.T. (°C)	Medium			
		Corn Meal	Seawater	Seaweed	Zobell's
23.11.75	4.6	57	43	34	580
31.10.76	5.6	105	209	97	2880
7.11.76	4.4	94	209	187	940
11. 5.76	12.5	311	6	89	780
11. 8.76	16.4	1572	1623	942	2940
13. 3.77	3.7	323	924	462	720

The 5 samples for each site and date were then compared between themselves using the Kruskal-Wallis one-way analysis of variance; this is a non-parametric test and thus does not depend upon any assumption about the distribution of the population. In all six cases an hypothesis of no difference between the samples was rejected at the 5% level, and with the exception of Newburn on 23.11.75, also at the 1% level.

For Newburn, 7.11.76 and Crimdon, 11.8.76 some results were obviously outliers but even tests carried out after their exclusion resulted in the rejection of the no difference hypothesis.

A parametric analysis of variance (ANOVA) was then used to examine the bacterial populations at each site separately.

It was apparent from the results (Table 11) that the hypothesis that the populations were different on the different dates was not acceptable at Newburn ($p>5\%$) but at Crimdon there was significant evidence of difference ($p<1\%$). The probability that the populations in the different samples were different was acceptable at both sites, with a higher degree of significance at Crimdon ($p \ll 0.5\%$). If the possible effects of season and temperature are ignored these findings suggest a more stable population at Newburn and if this is so it may well be related to the continuous supply of nutrients from the sewage outfall.

If, however, season and temperature are taken into account, it should be noted that, by chance, the Newburn site was only sampled in late autumn at temperatures between 4.0 - 5.9°C whilst Crimdon was sampled on two occasions in spring and once in summer, the temperatures at time of sampling varying between 5.5 - 14.7°C. Thus it is difficult to compare the two sites.

The information that these analyses of the bacterial counts yield can be summarised as:-

(1) The sample estimate of bacterial population was very probably of 'normal' distribution.

(2) Taking all samples (from both sites) into account there was

Table 11. Parametric analysis of variance of bacterial populations at the two sites

(DF - Degrees of Freedom; SS - Sum of Squares; SS% - Sum of Squares as % of total; VR - Variance Ratio; P - Probability)

NEWBURN	DF	SS	SS%	MS	VR	P(F≥VR)
Date	2	63.462	10.15	31.731	10.109	>5%
Date/Sample	12	241.383	38.62	20.115	6.409	<0.5%
Residual	102	320.158	51.23	3.139		
Total	116	625.002	100.00	5.388		
CRINDON						
Date	2	333.355	31.95	166.678	140.181	<1% (>0.5%)
Date/Sample	11	610.295	58.48	55.481	46.661	<< 0.5%)
Residual	84	99.878	9.57	1.189		
Total	97	1043.528	100.00	10.759		

true variation between the samples.

(3) That the isolation method was satisfactory and the quotation of a mean for the replicates is valid.

(4) That the populations varied significantly on different dates at Crimdon but not at Newburn.

(5) Although the experimental approach of sampling the two beaches on different dates meant that the beaches were sampled in different seasons of the year and at widely different temperatures, Table 9a makes quite clear that there was no marked difference in the numbers of bacteria counted from the two sites. The mean of the overall means for each date was 3.65 x 10^6/ g wet sand for Newburn and 3.30 x 10^6/ g wet sand from Crimdon. These figures are remarkably close.

Fungi

The dilution used for the fungal isolation plates was such that about 30% of the plates did not yield any colonies. The use of means for each sample was therefore not valid; instead figures are given for the total number of colonies for each set of plates of each medium.

Because there was thus no replication and given the constraints that the numbers are integers showing wide differences only non-parametric tests were used. The principal test used was the Kruskal-Wallis one-way analysis of variance. In 1971, Kent reported a detailed statistical analysis of fungal data, which was also later published, with biological implications, by Dickinson and Kent (1972). Kent tried a series of both parametric and non-parametric tests and concluded, for similar reasons, that parametric tests were unsuitable but that the Kruskal-Wallis test was valuable.

The data were first analysed to test if there was a significant difference between the populations from different samples or on differ-

ent dates. In the Kruskal-Wallis test the significance calculated from the circumstances should be below 0.05 if a difference between the samples is to be accepted. In no set of samples was this the case, the lowest significance calculated being 0.0035 with the other five sets being greater than 0.2. It can, therefore, be said that there was no evidence of differences in numbers of fungi between the samples.

Taking the two sites separately there was, at both, a highly significant difference between the three dates ($p \ll 1\%$). This finding is not in line with the results for bacteria. Although strictly speaking this finding precludes a statistical comparison of the fungal population on the two beaches the overall mean for Fenham (83) is much lower than that for Cullen (432).

The same test was applied to the data for numbers of colonies isolated on the different fungal media. The hypothesis of equal numbers was accepted and statistically these could not be distinguished but of these three media Czapek Yeast agar gave consistently the highest scores. One interpretation of this is that this medium, since it was enriched and non-saline, allowed fungi to grow which were orochthonous i.e. were not active or potentially active in the sediment.

The statistical analysis of the fungal data, therefore, indicates a relatively even distribution of fungi in the zones sampled with a significant difference in the populations on different dates which may have been seasonal or periodic variation or have been related to temperature.

Finally the bacterial data and the fungal data were compared using the Spearman Rank Correlation Coefficient. This test placed the pairs of readings (mean bacterial and total fungal counts respectively) in order of increasing bacterial counts. If the two were correlated the fungal counts could also be then expected to be in order.

The result of this exercise showed with a high degree of significance that there was an inverse correlation i.e. that when the bacterial count

was high the number of fungi grown was low and vice-versa. (Ranked correlation coefficient was minus 0.5019; significance 0.003). This relationship is also shown clearly if a scattergram of the values is prepared (see Fig. 11). There was thus very strong evidence of an inverse relationship between the numbers of bacteria and the numbers of fungi.

d. Qualitative Studies

Human faecal bacteria

A total of 225 McConkey agar plates had been inoculated from the samples and incubated at 37°C for 48 hours. The number of bacteria grown by this method was very much smaller than the number grown on Sobell's medium and only about 5% of these were pink colonies and therefore assumed to be lactose fermenters. These were tested by the methods described on p. 34 and none of them proved to be Escherichia coli (see Table 12). No other human faecal bacterium was identified. Three isolates of Klebsiella were obtained but these were identified as Klebsiella aerogenes and were probably of non-human origin.

Examination of the faecal stools collected showed that samples from the outside surface of the stool when cultured on horse blood nutrient agar and desoxycholate - citrate agar at 37°C yielded no bacterial growth. Samples from inside the stool, however, yielded a heavy growth of typical human faecal flora including the delicate Bactercides genus.

It is probable, therefore, that contact with seawater is lethal to the faecal bacteria and it is likely that, if this is a chemical effect, the effects of the more concentrated resting water of the beach will be greater than that of seawater itself.

The fact that faeces was not seen more commonly at the Newburn site was surprising but it was noted that when seen there had always been a

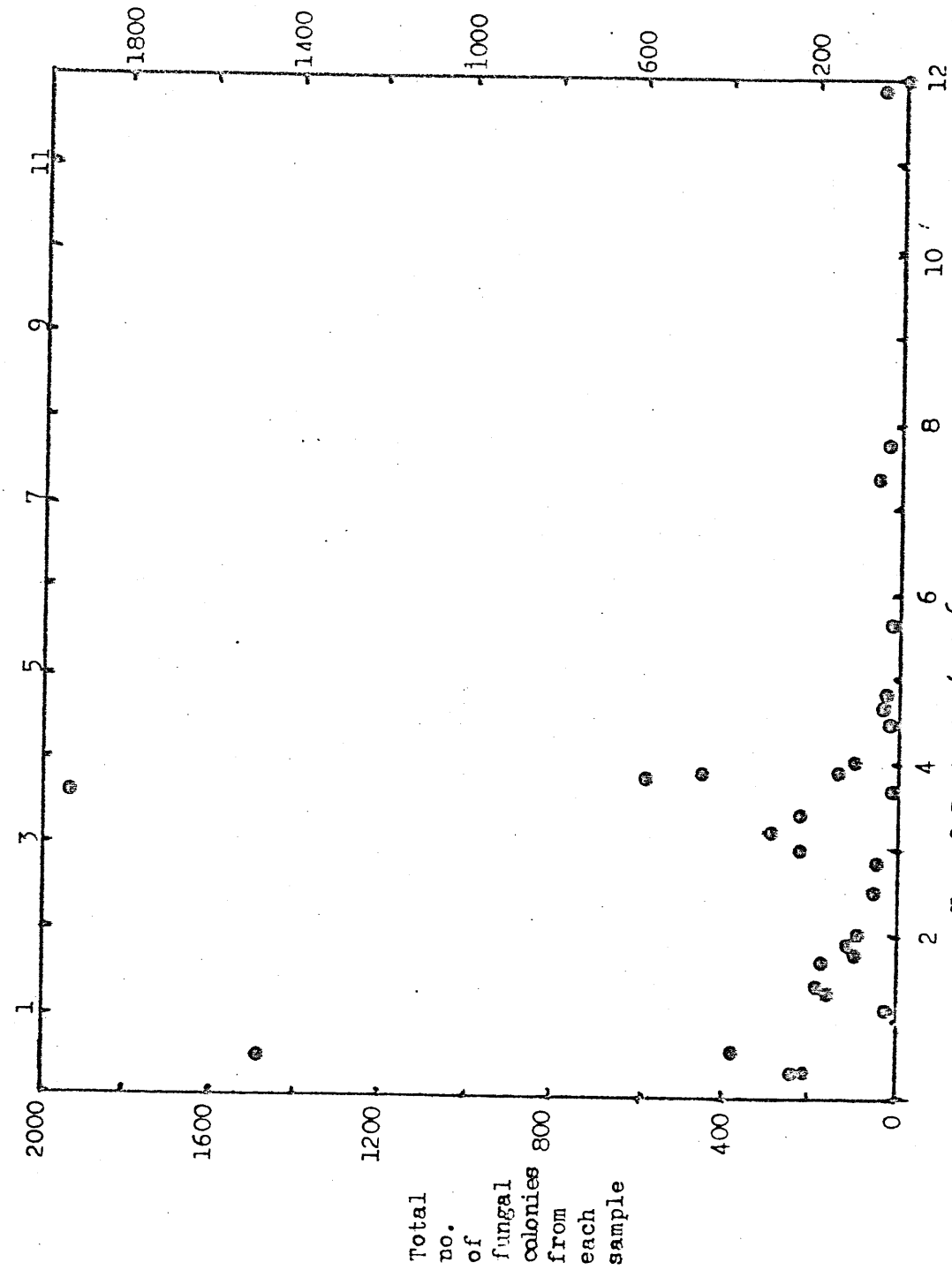


Fig. 11 Scattergram correlating total fungal colonies on all media with mean bacterial count from 29 samples of surface sand.

Table 12

Mean number of colonies isolated on McConkey agar from 5 samples of surface sand on three occasions from both sites: number of 'lactose-fermenters' (red or pink colonies); number of 'lactose-fermenters' identified as E.coli. (Incubation at 37°C for 48 h)

	NEWBURN			CRIMDON		
Date of sampling	23.11.75	21.10.76	7.11.76	11.3.76	11.8.76	13.3.77
Mean number of colonies on McConkey agar from five samples	52	63	71	32	41	73
Mean number of 'lactose-fermenters'	3	3	5	4	2	6
Number identified as E.coli	0	0	0	0	0	0

high tide and an on-shore wind which swept the floating stools rapidly on to the beach.

A factor affecting the contamination of the beach with faeces, which is not mentioned in the literature is the feeding habits of some seabirds. In some periods of the year the outfall was surrounded constantly with several hundred birds, mainly gulls, which were obviously feeding on matter coming from the outfall. It appeared almost certain that these birds were feeding, at least in part, on faeces and their numbers were sufficient to ensure that any visible, floating fragments were consumed within a few metres of the outfall orifice. These birds, when present, thus provided an efficient means of filtering-out any large particles of organic nutrient matter issuing from the outlet.

Other Bacteria

Examination of the plates of Zobell's medium showed that about 25% of the colonies were pigmented; of these 44% were yellow, 44% were orange, 8% were red. There were also occasional black colonies, usually tiny, and a few large green-yellow iridescent colonies. Approximately 2% of the colonies were agarolytic.

Flooding of some randomly selected plates with Kovacs reagent showed that between 80 and 85% of the colonies gave a positive oxidase reaction.

Gram staining of smears from a total of 300 randomly selected colonies indicated that 70% of the bacteria were gram-negative rods and most of these were small (<3µm long) and stained weakly with the counter-stain. The second most common (approx. 10%) morphological form was a slender, short gram-positive rod, some of which showed the 'Chinese writing' and palisade groupings typical of Corynebacterium whilst others showed spore formation. Also present were gram-positive cocci and a few organisms which showed only 'ghost' cells and apparently lysed forms

when smeared and stained. There were scanty colonies of yeasts present.

Twenty colonies from each sampling were subjected to the more complete testing described previously (Table 13). The most common genus was Pseudomonas (78/120), followed by Vibrio (12/120) and Flavobacterium (6/120).

The genus Pseudomonas is described by Gyllenburg and Hellund (1974) as the most significant group of the Pseudomonales concerned in decomposition. Some workers have placed those Pseudomonads which are completely inactive against sugars in a separate genus Gonmonas and if this separation is accepted then the majority of Pseudomonads isolated in this work should be so labelled as they showed no such activity. Similarly nearly all of them failed to produce either pigment on the medium used or arginine dihydrolase, both of which are accepted as strong indicators of the genus Pseudomonas. However, computer-composed 'identification' codes of these organisms indicated, mainly on negative findings, that they were either Pseudomonas, or with a lower probability, Achromobacter. The taxonomic position of Achromobacter is confusing and the problem of separating isolates from Pseudomonas is discussed in detail by Ingram and Shewan (1960). These workers believed that many achromogenic Pseudomonads have, in the past, been referred to as Achromobacter.

Species identification of Pseudomonads was not attempted. Ingram and Shewan (1960) have stated that the more experienced workers in the marine field have, for the present, ceased trying to identify separate species of this genus.

Many of the strains examined could reduce nitrates and though not useful for classification, at present, this is of interest since some Pseudomonads are believed to use denitrification as an alternative anaerobic mechanism for respiration.

The ecological significance of the fact that the large majority of the bacteria grown could produce cytochrome oxidase is not clear since the physiological function of this enzyme is not fully understood.

Table 13. Bacterial genera identified from surface sand of two beaches

(Numbers are records out of 20)

NEWBURN

	23.11.75	31.10.76	27.11.76	Total (No/60)
Pseudomonas	13	12	12	37
Flavobacterium	2	1	1	4
Corynebacterium	1	-	1	2
Chromobacterium	1	-	-	1
Vibrio	1	2	3	6
Micrococcus	-	1	1	2
Xanthomonas	-	-	1	1
Bacillus	-	1	-	1
Achromobacter	-	1	-	1
Unknown	1	2	1	4
Yeast	1	-	-	1

CRIMDON

	11.5.76	11.8.76	13.3.77	Total (No/60)
Pseudomonas	13	14	14	41
Flavobacterium	1	2	1	4
Corynebacterium	1	-	1	2
Vibrio	3	1	2	6
Micrococcus	1	1	-	2
Xanthomonas	-	1	-	1
Klebsiella	-	-	1	1
Achromobacter	-	1	-	1
Unknown	-	-	1	1
Yeast	1	-	-	1

Flavobacterium is a vaguely defined genus and it is possible that some of these isolates should be called Cytophaga. Some of the Corynebacteria isolates could possibly have been classified as Arthrobacter.

Although only one Bacillus was present in the randomly selected 120 isolates the frequency of the genus was probably greater than this. Assuming identity from colonial appearance they appeared to represent about 3% of the total population.

Fungi

On the first sampling from each site every colony grown was examined and an attempt made to identify it to species level. Newburn showed the more diverse flora, yielding 22 genera as compared with the 7 from Crimdon (Table 14).

The colonies isolated from subsequent samplings were not individually examined but representatives of the most commonly occurring colonies were identified and estimates made of their prevalence. Species of Penicillium, Cladosporium, Aspergillus and a black unidentified hyphomycete proved to be the most commonly isolated genera from both sites, with Acremonium and Phoma also being very common. The majority of fungi were, therefore, Hyphomycete genera normally considered to be 'terrestial'. Of specific interest was the isolation of the basidiomycete Sistrotrema and the filamentous yeast Trichosporon.

The results as a whole confirm the findings of previous work on similar habitats. Cladosporium, Stemphylium and Alternaria were commonly isolated from sandy beaches subject to salt spray or immersion by high tide waters by Nicot (1958a,c) whose published list shows one ascomycete, 36 species of Fungi Imperfecti and two sterile species. Brown (1958b) frequently isolated Penicillium, Cladosporium and Verticillium from the 'open sands' of tide-washed beaches.

Table 14. Occurrence of fungi in surface sediments of Horburn (25.11.75) and Grirlon (11.5.76) expressed as a percentage of total isolates

Genus	Occurrence (%)	
	Horburn	Grirlon
Acremonium	2	-
Alternaria	<1	-
Arthrinium	1	-
Aspergillus	<1	46
Aureobasidium pullulans	3	2
Botrytis	1	-
Cephalosporium	6	-
Cladosporium cladosporioides	29	3
Cladosporium herbarum	13	-
Cladosporium macrocarpum	<1	-
Cylindrocarpon	<1	1
Dematiaceae	<1	-
Fusarium	<1	-
Geotrichum	<1	-
Harzania	<1	-
Penicillium	16	20
Phialophora	<1	-
Phoma	5	-
Scopulariopsis	2	-
Sistotrema	3	-
Stemphylium	1	-
Thielaviopsis	<1	-
Tilletiopsis	2	-
Venturia	<1	-
Verticillium	1	-
Unidentified black hyphomycete	-	24
Sterile forms	5	4

The total number of species isolated from the tidal areas of both bay sites was 12.

It is important to note that all the common genera isolated in this work proved able to grow and sporulate on the seawater medium. Since it is certain that many of the colonies arose from spores these fungi were obviously also able to germinate on this medium. Thus although they were members of genera normally considered as terrestial the species could go through normal life cycles at a salinity equal to that of seawater and supported only by the dissolved nutrients in that water. It is also possible that some of those species which did not spore on the seawater media would do so at those points on the beach where salinity was rapidly reduced by capillary rise of fresh water.

iv STUDIES ON ADSORPTION

(a) Cultures of deionized water washings of fresh sand yielded 46% more colonies than could be grown from seawater washings (Table 15). If it is accepted that a percentage of the bacteria removed from the sand by the deionized water were killed or injured by the non-saline conditions (and, according to the results of Khiyama & Makemson, 1975, when comparing numbers grown on distilled water media and seawater media, this percentage could be over 90) then it is clear that freshwater removed many more bacteria from the surface of sand grains than seawater did.

This confirms the results of Anderson and Meadows (1969) who claimed this was an hitherto unreported finding. However, Wagner and Schwerta (1961, 1963) had demonstrated that transport of bacteria through sandy sediments was affected by salinity and that a higher number of cells

were retained under marine conditions than under limnic conditions.

(b) 10 replicate viable counts from a freshly collected seawater sample gave a mean bacterial count of 564 x 10^3/ cm^3. In all cases this count was significantly reduced by passage through a 10 cm column of sterile sand (Table 16).

The mean count after passage through these columns was 220 x 10^3/ cm^3 and these results, therefore, show that 60% of bacteria from the percolating seawater were retained, presumably by adsorption to particles, by a 10 cm layer of surface beach sand.

(c) This experiment was designed to show whether the reduction in bacterial count after passage through a column of sand, demonstrated in experiment (b), was a generalised reduction or whether different species were affected differently.

The data showed that the two species of Pseudomonas were almost totally adsorbed on the sand column, with the count being reduced to less than 0.5% of the original (Table 17).

The Bacillus species passed through easily and the count was only marginally reduced i.e. not more than could be expected from dilution with the interstitial water.

Corynebacterium and Micrococcus were both reduced in count to less than 20% of the original (18% and 12% respectively).

(d) If a normal tide is observed it will be seen that, unless the beach is very steep, the sand is first covered by thin layers of water which disturb it very little. Some of the water soaks into the sand, driving air out; some retreats to the waters edge gently swirling the sand as it does so. The sand is then pounded heavily by wave action until the breakers pass the observed point, when, until the ebb tide, the sand is covered by comparatively still water. As the tide ebbs the process is reversed and heavy pounding of the sand is followed by gentle mixing.

This experiment was devised to imitate this tide cycle. The results

Table 15. Mean number of colonies cultured from the last 3 of 8 aliquots of 20 cm^3 of seawater or deionised water after passage through ten 10 cm long columns of freshly collected sand

Sand Sample	1	2	3	4	5	6	7	8	9	10	Overall Mean
Deionised water	62	64	56	51	68	72	50	67	62	73	63
Seawater	44	48	45	39	36	51	42	40	34	49	43

Table 16. Bacterial counts from seawater samples after passage through sand columns and the percentage reduction in count from the original figure

Sand sample	1	2	3	4	5	
Count	138	156	252	384	282	x 10^3/ ml
Reduction (%)	75.5	72.3	55.3	31.9	50.0	

Sand sample	6	7	8	9	10	
Count	192	144	180	246	228	x 10^3/ ml
Reduction (%)	65.9	74.5	68.1	56.4	59.6	

Table 17. Mean colony counts of saline suspensions of five bacterial species before and after passage through a 10 cm column of sterile wet sand

	Original Count	Count after passage through column
Pseudomonas (A)	218	0
Pseudomonas (B)	180	1
Bacillus	232	191
Corynebacterium	213	38
Micrococcus	198	24

(Table 18) showed that the first gentle mixing of sand and water released only small numbers of bacteria and that this number increased sharply with more vigorous mixing.

A two hour period of standing did not reduce these numbers which indicated that there was no settling of the organisms.

The second vigorous mixing, equivalent to the ebb tide, showed no significant change in numbers of bacteria and the final gentle mix showed no significant change in levels. It had been thought possible that there might be some readsorption of bacteria in this phase but there was no evidence of this.

(c) The bacteria present in wet sand are either 'free' in the interstitial water or are attached to the sand grains. Khiyama and Makemson (1973) found that the number of bacteria per cm^3 of interstitial water accounted for 22 - 46% of the total number of bacteria in each g of wet sand. This manner of expressing the facts gives a false impression of the number of unattached bacteria since the volume of water present is only about one third of the total volume of wet sand.

If the total numbers of bacteria present in wet sand samples are counted and the same samples are then thoroughly washed in sterile sea-water to remove interstitial bacteria and the counts repeated the difference in the numbers per g of wet sand gives an estimate of the percentage of bacteria attached as compared with those free (Table 19).

The data obtained in this experiment indicated that, on this occasion, about 75% of the bacteria present were attached to the grains leaving 25% free in the interstitial water.

If these results are expressed in the manner used by Khiyama and Makemson (1973) they would indicate that the number of bacteria per cm^3 of interstitial water were about 75% of the total number of bacteria in each g of wet sand.

Table 18. Mean number of colonies grown from duplicate samples of the supernatant seawater obtained after 5 sequential treatments of 10 fresh sand samples

Sand sample	1	2	3	4	5	6	7	8	9	10	Mean
Treatment:											
Gentle mixing	23	5	3	12	9	18	21	9	4	2	11
Vigorous mixing	88	90	29	105	44	161	142	150	112	70	99
After 2h undisturbed	86	92	56	110	42	170	138	114	105	72	98
Vigorous mixing	78	88	69	121	37	163	141	153	103	74	103
Gentle mixing	86	78	53	132	46	180	140	160	138	71	108

Table 19. Mean viable counts of bacteria ($\times 10^6$/ g wet sand) from 5 samples of fresh sand and from the same sand samples after washing with 10 x 100 cm^3 sterile seawater

Sample	1	2	3	4	5	Mean
Fresh sand	2.50	5.18	2.68	5.54	4.68	3.32
Washed sand	1.82	2.32	1.90	2.68	3.64	2.47
Difference	0.68	0.86	0.78	0.86	1.04	0.84

v. STUDIES ON DESICCATION

The number of viable bacteria fell quickly in the first 5 d of desiccation but then reached a level which diminished only slowly over the next 23 d (Fig. 12). At the end of this period there were still an appreciable number of viable bacteria. This pattern was the same at all three temperatures (4°, 20° and 37°C) but the number of bacteria surviving varied inversely with the temperature.

Gram staining and colonial appearance of those organisms growing on the final (28 d) plates indicated that the desiccation process had selected at least sixteen species and that these were gram positive spore-bearing rods and gram positive cocci. The only gram negative rod surviving was a large one which was grossly pleomorphic and which included forms which could possibly have been spores.

Although the self-indicating silica gel retained its blue colour throughout the experiment, indicating adequate sealing of the desiccant dishes and an ample reserve of 'drying-power', it was thought possible that water could have been retained in the cracks and fissures of the surface of the sand grains.

To check this, five samples were accurately weighed after 28 d desiccation at 37°C. They were then placed in a hot air oven at 102°C for 18 h and, after cooling, reweighed. All the weights were slightly reduced by this process with the mean reduction in weight being 0.106%.

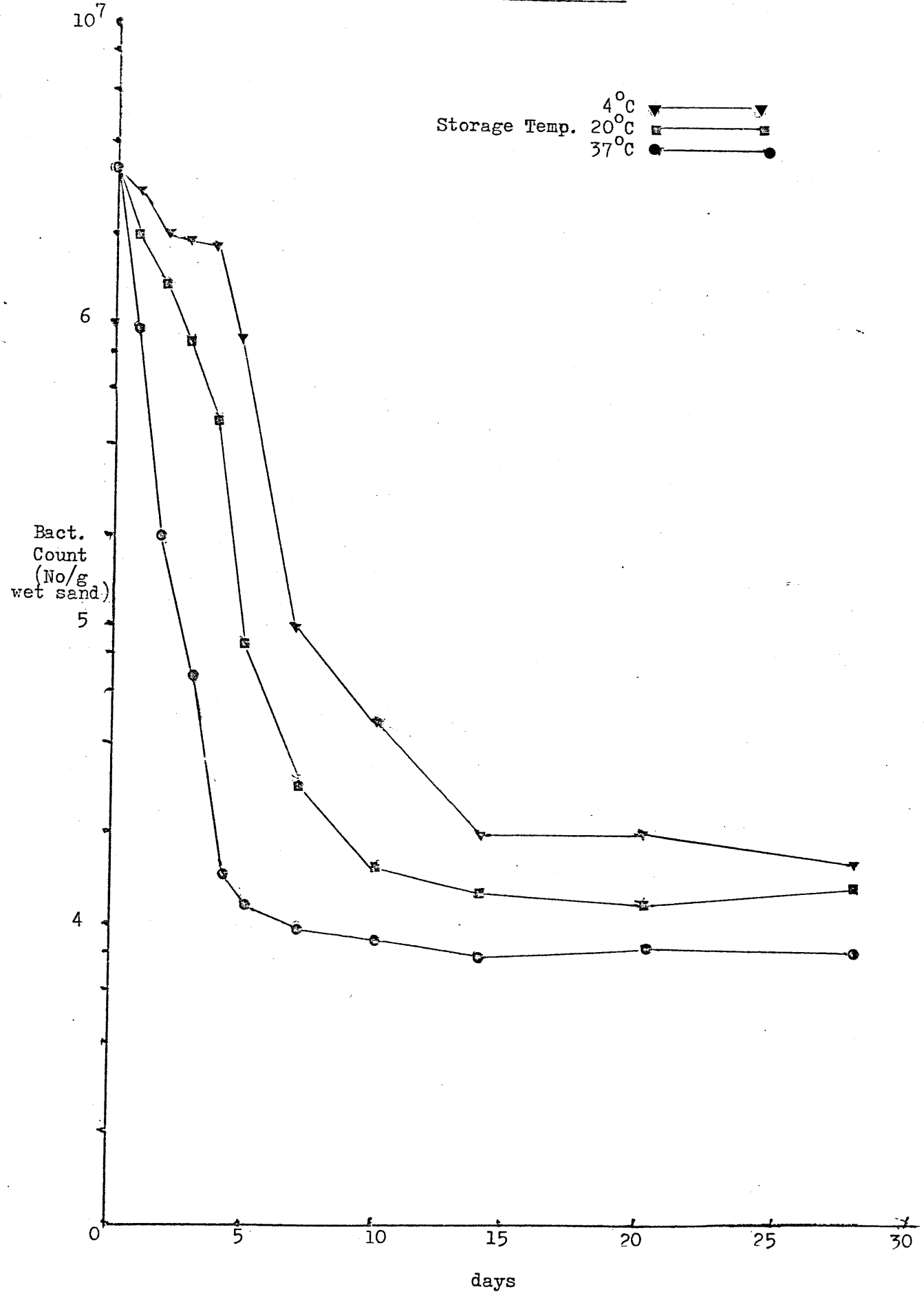
It is assumed, therefore, that the sand had retained Ca. 0.1% of its own weight of water after 28 d drying at 37°C.

These results suggest that if the surrounding air is completely dry it will result in death of the large majority of gram negative bacilli in sand within 5 d. The sand will, however, retain a small amount of moisture for at least 28 d at 37°C and this is accompanied by the survival of a population of spore-bearing bacilli and gram positive cocci.

In the intertidal zone it is unlikely that such dry conditions are ever obtained. The surface of the sand may reach higher temperatures than 37°C during summer sunshine but there will always be water vapour present arising from the water table below.

In the surface layer of sand outside the regularly inundated intertidal zone, however, drying must be an important factor in changing the bacterial flora of the sand.

Fig. 12 Bacterial numbers after increasing periods of desiccation



C. D I S C U S S I O N

These studies of the microbial populations of the intertidal zone of two sandy beaches on the North-East Coast of England were initiated with the intention of showing what effects, if any, the direct pollution of a sandy beach with sewage had upon the microbial flora of that beach. Newburn beach was chosen as a good example of a grossly polluted beach and Crimdon as a beach which was apparently essentially similar but which was not directly polluted. Early perusal of the literature, however, indicated that there was a shortage of information on the microbial flora to be expected in the intertidal zone of sandy beaches and therefore the primary aim became that of establishing what were the characteristics of the bacterial and fungal populations present in this zone and of demonstrating any relationship that existed between the two. It was hoped that these studies would also then reveal either that there were differences between the two beaches attributable to pollution by sewage or that such pollution had no appreciable effects.

i. PHYSICAL AND CHEMICAL ANALYSIS

It was first necessary to study the physical and chemical aspects of the two sites. Both beaches proved to be sediments of mainly medium sized grains of siliceous sand. The grain-size distribution confirmed the visual evidence that Newburn had been a beach of fine sand from which the finer grains were being lost, whilst Crimdon, as indicated by its slope, had been a beach of somewhat coarser sand upon which finer grains were being deposited.

The two most comprehensive studies of the physical characteristics

of beach sand were carried out some decades ago. Bruce (1928) showed that the size of grains and the degree of their sorting are directly related to the water retaining capacity of sand. Using graded sands he reported retention figures of 35.8 vols. - 44.7 vols. of water/100 vols. of wet sand with the volume of water retained increasing as the grain size increased. However, in a short series of tests on natural (i.e. ungraded) sand he found the volume of water retained to be only 29 vols. i.e. less than the volume retained by the finest grade of sand. Bruce had calculated that the theoretical pore space of the graded sand was 26% and explained measured values over 10% higher than this by stating that "sand grains do not readily fall into the position of closest contact but rather into stable arcades".

Webb (1958) also discussed the calculation of pore space and the measurement of water retention of sand at great length. He pointed out that the porosity of sand depends not only upon the size of the grains but also upon their shape. Larger grains were shown to be more angular than small ones and he, too, was able to show that a mixture of grades retained less water than a single grade of sand of even the smallest grain size.

In their discussion of this problem neither Bruce (1928) nor Webb (1958) took account of the numerous crevices and fissures in the surface of a grain which must significantly increase the space available for water.

The first method used for estimating water retention in this work was to measure the volume of water necessary to saturate a given volume of dry sand and Webb (1958) has stated that this is also the most accurate means of determining pore space.

The measurements carried out have shown that dry surface sand from these two beaches could absorb approximately 30 cm^3 of water/100 g sand and could retain this against the force of gravity (but not necessarily against the force of capillary attraction). Naturally wet sand, after

overnight settling, contained 33 cm^3 of water/100 g, this higher figure probably being due to the fact that the method did not allow drainage to occur. When air was forced through the sand columns only about one third of this water was removed and it is contended that only this removable water was 'free' to drain into the deeper layers with the rest being held as a film on the surface of the grains where it could only be lost by evaporation.

Evaporation was not measured in this work but Bruce (1928) and Webb (1958) both found that the evaporation rate at a sand surface was about the same as that from a free water surface. However, Bruce (1928) postulated that the internal surface of the sand contributed little to total evaporation loss, the actual escape of water molecules being limited by the rate of diffusion through the interstices. Both these workers found that it took 30 - 48 h for a 5 cm layer of sand to lose its water by evaporation and Webb (1958) showed that a mixed grade sand would hold 7.5% of its water for at least this period. Bruce stated that evaporation rate was unaffected by grain size but Webb disagreed since he found there were significant differences between different grades of sand which only became obvious during the 24 - 48 h period of exposure to air.

The flow-rate results (Table 2) show that water flows rapidly through the top 5 - 10 cm of sand. Again it is probable that the exact rate is determined by the grain size, shape and sorting i.e. the porosity of the sand. The less well sorted the sand the slower the flow-rate, probably due to the 'tortuosity' factor. No significant difference in the rate of flow of seawater and distilled water was demonstrated and this fits with the findings of Bruce (1928) who could show no difference between the capillary rise of fresh and seawater through sand.

These factors retention, evaporation and flow, will directly effect the water content of surface sand between tide cycles and the experiments on water content show that when surface sand is immersed twice daily the

loss of water during the period of emersion is minimal, even at relatively high temperatures.

The salinity of the interstitial water of the surface sand, was slightly higher than that of the adjacent open sea. These samples were taken immediately after the tide had uncovered the beach and according to Bruce (1928) salinity is maximal at this time. He showed that it then progressively fell, due to capillary rise of fresh water, until just before the sampling point was immersed again, when it rose to seawater levels. The degree of diminution was proportional to the distance of the sampling point above M.W.

Johnson (1967) found that interstitial salinities remained fairly constant in the upper layers of beach sand during the summer. High on the beach evaporation could increase interstitial salinities during prolonged exposure. Winter increased the flow of fresh water and caused variation at beach elevations above 1 m. Below this elevation salinities varied less than in the open sea.

There was true variation in the salinities measured in this work; this was minimal at Newburn, a flat beach, but was more marked at Crimdon which has a slope. The variation may thus have been related to the elevation at which the samples were taken.

Salinity should be defined as the percentage of total dissolved salts in water. To calculate it exactly, therefore, the levels of all the major salts present should be measured. Since this is laborious it has usually been measured by estimating chloride levels and then either multiplying by a factor or referring to tables such as Knudsen's tables. The former method has been used in this work to give a salinity figure. However, estimation of sodium and potassium has also been carried out and the variation in these indicates that the use of chlorinity alone is not a truly accurate means of estimating salinity. For biological purposes it is often the osmotic effect of 'salinity' that is important and for this purpose the measurement of osmolality would be a more accurate and

useful value. Using an osmometer, this value can be obtained easily and quickly by measurement of freezing point depression and, particularly in polluted areas, would rapidly reveal an unexpected addition to the solute level not shown by changes in chlorides.

Since this work was completed a few random samples have been tested in this way and gave values of 1000 milliosmoles on average suggesting that at these sites, in spite of pollution, there were no 'abnormal' levels.

The small series of magnesium measurements showed complete uniformity with a lower value occurring at Crimdon.

The bicarbonate levels were too low for satisfactory estimation by the method employed, being at the limit of its sensitivity range. However if the level measured (2 mmol/l) is compared with that reported for seawater by Rheinheimer (1974) who quoted 0.145 g/kg which is equivalent to 1.7 mmol/l, it can be seen that the levels present were probably almost the same as the seawater levels.

Urea was undetectable on both beaches and it must be assumed the urea present in the sewage at Newburn was broken down, at least to ammonia, by urea-splitting organisms present in faeces, before the sewage reached the beach.

The organic carbon levels showed a higher level at Newburn, which is attributed at least partially, to the higher percentage of organic fragments shown to be present and this in turn is attributed to the presence of the sewage outfall. The apparent discrepancy between difference in organic particle counts and difference in organic carbon levels is probably artificial since organic particles are significantly lighter than the siliceous grains. Seacoal was shown to have contributed only slightly to the measured levels. This does not necessarily mean that its contribution as a nutrient was insignificant.

The carbon levels from both sites were much higher than the majority of levels recorded by Pugh *et al.* (1974) although some of the

levels obtained on their 'muddy' beach at Llanfonna were similar. These workers had washed their sand in fresh water before estimating carbon and it seems likely that this would lower levels (see later discussion on removal of bacteria by freshwater). However, it should also be noted that both Crimdon and Newburn sediments contained a significant percentage of organic fragments. This percentage is probably higher than would be found on 'clean' beaches but one cannot be sure of this since few workers record the number of organic particles present in sand. This is surprising since this easily ascertained fact is a valuable datum.

A number of workers have studied the levels of organic carbon in marine deposits and the results of this work have been summarised by Newell (1970). There seems to be ample evidence from these results to support Newell's statement that the percentage of organic carbon is inversely related to the size of the particles. There is, however, some disagreement about the source of the organic carbon present. Newell (1970) stated that bacterial carbon might be sufficiently plentiful to account for a large proportion of sediment carbon and points out that if an assumption is made that a layer of bacteria surrounds each particle and a line is then calculated relating bacterial carbon to mean particle diameter then that line is very similar to the results obtained from his own laboratory-cultured deposits (Newell, 1965) and also those obtained from the North Kent Coast by Longbottom (1968). However, Dale (1974) has calculated that only 1.2% of the organic carbon of sediments is bacterial carbon and Hargrave (1972) calculated that less than 1% of the surface area of sand is covered by bacteria. Both these workers, however, admit that there is a definite relationship between organic carbon in sediments and the number of bacteria present.

Steel and Baird (1968) postulated that nearly all the organic matter on a sandy beach was present in forms attached to the sand-grains but stated that "the low productivity of the sand could mean that the

small proportion of unattached organic matter is important in the interstitial food chains if it has a rapid turn over". They also noted the presence of some weed debris in the sand and concluded that this was "an indication of an external source for some of the unattached material."

Although none of these workers seem to have examined sands of >350 μm diameter, if Newell's line relating organic carbon to particle size (Newell, 1970) is extrapolated to 450 μm (the median particle size at Newburn and Crimdon) then the corresponding level of organic carbon should have been undetectable. Both Newell (1965) and Longbottom (1968) showed that levels similar to those found at Newburn and Crimdon were related to sand with a particle size of only 120 μm. This indicates an 'excess' of carbon on both these beaches which must, therefore, come from an excess of bacteria (Newells theory) or to an excess of other organic matter (see Steel and Baird, 1968 and Dale, 1974).

At these sites it is likely that this 'excess' was due to unattached organic material derived from sewage and the storm drain at Newburn and perhaps from detritus carried onto the Crimdon beach by the stream.

It is also possible that carbon was present in the form of highly refractile humic acid compounds (see Dale, 1974).

The nitrogen levels found were similarly higher than would be expected from the results of other workers and the low C/N ratios suggest that this would be due to the presence of nitrates, nitrites and, in the case of Newburn, possibly ammonia.

iii MICROBIAL POPULATIONS

It is evident from my results (section F iii) and other work that the intertidal zone of the sandy beach has a resident population of bacteria and that this population is a diverse one. The methods used here have been such that only the heterotrophic part of this population has been examined. The results of other workers have indicated, for example, that there is, probably, a similarly diverse population of autotrophic bacteria present (e.g. Eltringham, 1971). Certainly the inorganic substances these organisms utilize are present in more than adequate amounts. Whether the auxins or growth factors that are needed by many of them (the auxotrophs) are also present needs to be ascertained.

Only the surface layers of sand have been examined since the majority of heterotrophs present are at the surface where organic material is deposited. However, there is no evidence to show that the autotrophs are similarly concentrated in the upper layers. Indeed, as Eltringham (1971) has pointed out, organisms such as the sulphur bacteria, which play a significant part in primary productivity, are concentrated in the black 'sulphide' layer, which in regularly disturbed sandy beaches is at some depth. Similarly strict anaerobes will presumably be restricted to the deeper, non-aerated layers. However, Zobell (1946) and Wood (1955) have stated that strict anaerobes are rare in the marine environment.

The so-called higher bacteria (actinomycetes and streptomycetes) have not been specifically searched for either, although some of the organic fragments examined did show the presence of branching organisms which seemed to be much finer than the hyphae of fungi and scanty colonies of a streptomycete were grown on the fungal media. Hunn and Shephard (1946) isolated agar-digesting species of actinomycetes from intertidal sediments and Chesters, Apinis and Turner (1956) showed that Actinomyces and Streptomyces were active in the decomposition of cast-up seaweed.

It is probable, therefore, that they play some part in the breakdown of small seaweed fragments present in the sand.

The numbers of bacteria present in intertidal sediments generally seems to be directly related to the surface area of particles available, either for colonisation and/or for adsorption of nutrients (see e.g. Newell, 1970, Hargrave, 1972 and Dale, 1974). This surface area is governed by particle size principally but a factor that seems to have been ignored is the amount of scoring, cracking and fissuring shown by particle surfaces. This could significantly increase the surface area and can by no means be regarded as a constant since it will depend, to a large extent, on the hardness and brittleness of the particles which will in turn depend upon their geological source. The population size is also believed generally to be related to nutrient levels (see Newell, 1970, Bianchi, 1973, and Dale, 1974) and these in turn are generally related to surface area although my results have indicated that the presence of particulate organic debris can upset this relationship (see previous discussion p. 102). The calculated relationship between particle size and bacterial numbers would indicate that when the particles reach a large size then bacteria are no longer present in significant numbers. That this is not so is indicated by the work of Batoosingh and Anthony (1971) who have shown that there are significant numbers of bacteria attached to the surface of 'particles' as large as pebbles.

The statistical analysis of the bacterial numbers recorded by me have shown that there was variation between samples, taken at the same time, at stations only 10 m apart but that there was no marked difference in levels from season to season (temperature to temperature?). There was, and this would accord with the results of Andrews, Floodgate and Pugh (1976), some indication that numbers were lower when samples were collected in hot weather. This may be a desiccation effect or, and this is more likely, a reduction due to exposure to sunlight.

Comparison of the numbers found with those of other workers is

almost impossible because of the different methods used to remove bacteria from sand; the division between direct and viable counting methods; the different media used for viable counts; the different temperatures of incubation and perhaps most important, the diversity of modes of expression of bacterial numbers.

These numbers have been related to volume of dry sand and to volume of wet sand; to weight, of dry or wet sand; to calculated surface area of particles and to volume of associated water. Andrews, Floodgate and Pugh (1976) discussed this problem and concluded that the number of bacterial units was best related to ml of 'associated water' mainly on the grounds that this unit was "better than other alternatives in any comparison with the enumeration of bacteria in the seawater which is normally and naturally expressed as bacterial units/ml."

Anderson and Meadows (1969), however, had strongly supported the expression of bacterial units/unit surface area of particles since they felt that relating numbers to volume or weight of dry or wet sand could give 'misleading' figures for the numbers of bacteria present. They did not consider the mode of expression used by Andrews, Floodgate and Pugh (1976).

I disagree with both of these views. The term used by Andrews and his colleagues has, as they admit, a moisture content dependence which, whilst not causing problems on their model beach, does prevent any comparison with reports of other workers unless those workers estimate water content on each sample of sediment from which bacteria are counted. A very low water content can also cause gross distortion of counts from samples collected in the field as is seen in at least one of the results obtained in previous work (Pugh et al, 1974).

The relation of bacterial numbers to surface area as recommended by Anderson and Meadows involves using a unit the calculation of which is based upon a supposition that all the particles are spheres, which immediately introduces what could be a significantly large error. It

also assumes that the relationship between the number of bacteria adsorbed to surfaces and the number 'free' in the interstitial water is a constant and there is no evidence for this.

My own view is that numbers are best related to either weight or volume of wet sand since the units of weight or volume are at least absolute and accurately measurable and to my mind, their use conveys most realistically the correct impression of the total number of bacteria present. A gram of wet mud could have ten times more bacteria present than a gram of relatively dry sand, and yet Anderson and Meadows' mode of expression and that of Andrews and his colleagues could give the impression that the numbers present were equal.

Using weight or volume relationships the factors that influence the numbers present in a unit need, of course, to be kept in mind.

The samples examined by me were all taken from the mid-line between the HWS and the LWS of the tide on the day of sampling and no attempt has been made to study the numbers down a transect of the beaches. However, this had been studied by Westhoide (1968), Rheinheimer (1974), Pugh et al. (1974) and Andrews et al. (1976) on their model beach.

The overall impression from the findings of these workers is that numbers decrease down the intertidal zone. However, this is an oversimplification. From natural beaches Pugh et al. (1974) and Anderson and Meadows (1969) found their highest numbers occurred in the upper part of the beach; Perkins (1974) stated that the microbial flora was sparsest at the HWS; Rheinheimer (1974) postulated that counts were always lowest in those parts of the beach where the sand was most often disturbed. Andrews, Floodgate and Pugh (1976) suggested that the high level of bacteria found at a station on the lower part of their model beach, was due to the presence of fine particulate organic matter filming the deposit at this point, but, had been unable to correlate bacterial numbers generally with any of their chemical findings; Rheinheimer's diagram (1974) clearly indicated a correlation between bacterial numbers

and those points on the transect where litter would normally be deposited.

The population of heterotrophic bacteria isolated in this work was definitely 'marine' from the point of view of genera present, with a definite predominance of Pseudomonads. These Pseudomonads have been ascribed to the Pseudomonas but the identification is definitely tentative and indeed could not be otherwise in the present state of classification of marine bacteria. For example, organisms which seem very similar were placed by Wood (1965) into the genus Mycoplana. He described this group as the most important single group of bacteria found in the sea and stated that they were normally ascribed to Achromobacter or Flavobacterium. He suggested that this genus bridged the gap between the pseudomonads and the corynebacteria. Kriss (1963), however, has stated that the majority of bacteria present in the North Sea were Pseudomonas and Ratcosingh and Anthony (1971) found that the majority of the bacteria attached to pebbles were also Pseudomonas.

The role of bacteria in intertidal sediments appears to be that of transformers of materials. The heterotrophic bacteria are consuming organisms, breaking down the variety of particulate organic material left as detritus in the surface sand by receding tides and producing soluble nutrients which are washed back into the sea. The autotrophs are producers of organic material using the large supply of inorganic substances available. All animals of the shore, whether detritus feeders or carnivores are ultimately dependent upon the activities of the bacteria with the exception of those that feed directly on diatoms (Perkins, 1974).

The bacteria may also have physical effects on the sand. Rheinheimer (1974) has stated that they may, when they colonise particles, change their size and shape and that their slime production can cause aggregation of smaller particles. He suggested too that bacterial fimbriae could cause particles to adhere.

It is unlikely that the methods used in this work have isolated all the fungi present in the sediments. Brown (1958a) found, using impression slides, that mycelium was often present in sands which appeared to be sterile on culture.

No 'bait' techniques were used in this work and therefore aquatic zoospore - producing fungi may have been missed, although it could be said that the organic fragments present in the sand samples tested had already acted as baits.

However, the major species isolated were those that have been commonly isolated by other workers from coastal waters and sediments. Rhode (1954) stated that Penicillium and Aspergillus were the commonest fungi in dune soils. Penicillium and Cladosporium were frequently isolated by Brown (1958b) and she pointed out that although Cladosporium is a common aerial contaminant its frequency and distribution on her soil plates strongly suggest it was an active member of the flora. Ahearn and Meyers (1976) gave the following list of percentage frequencies for selected moulds in estuarine waters:- Cladosporium 97; Penicillium 80; Cephalosporium 49; Fusarium 25; Aureobasidium (Pullularia) 72.

The fungi that have been isolated from these sediments thus appear to be predominantly 'terrestrial' forms. Perkins (1974) divided them into 'salt-marsh' species and 'transients'. He stated that the salt marsh species occurred more frequently on the upper region of the intertidal zone whereas the more widespread and transient species occurred more frequently on the lower shore.

He explained this difference as being due to the 'transients' being washed from the land and finding favourable conditions for growth among the non-specific detritus low on the shore whilst the upper shore was colonised by fungi adapted to live on salt marsh plants.

Litchfield and Floodgate (1975) when examining Irish Sea sediments were surprised to find that the dominant organisms in some of their cores

were fungi of the genera Cladosporium, Penicillium and Fusarium. Having confirmed by further tests that these fungi were not contaminants they speculated that such 'terrestial' fungi may have an importance greater than is generally accepted.

The most interesting part of Perkins' hypothesis (1974) is the idea that the 'transients' find favourable conditions on the lower shore. This implies that these species are not merely halotolerant but halophilic.

A considerable amount of work has been done trying to answer the question "What is a marine fungus?" and the viewpoint of the various workers has varied widely. However, there has been a tendency to restrict the term 'marine' to aquatic fungi obliged to live in saline conditions and other fungi present in marine situations have been dismissed as 'terrestial contaminants'. This view ignores the ability of some species of such genera as Penicillium, Aspergillus and Cladosporium not merely to survive in marine conditions but to flourish. Jones (1976) has published a table, culled from the reports of some dozen authors, which indicates the response of 'terrestial' fungi to salinity. This table shows quite clearly that whilst many species grow better in limnic conditions and others grow equally well in saline or limnic, there are some species of Penicillium, Aspergillus, Phoma, Cephalosporium, Fusarium, Aureobasidium, Stemphylium and Geomyces which show increased growth in seawater.

This response to salinity is not a simple one and may be governed by other factors such as the nutrients available and the ambient temperature.

Ritchie pointed out in 1959 that some fungi, and he gave Phoma as one example, could grow better at high salinity if the temperature was high, and better at low salinity if the temperature was low. This 'Phoma pattern', as it has been called since, indicates that for some fungi at least 'optimum' salinity is a fluctuating value shifting as temperature shifts and Ritchie believed that this relationship between temperature

and salinity was a function of osmotic pressure.

Such a pattern could clearly account for the apparent 'seasonal' increase in numbers of fungi indicated by this work.

Borut and Johnson (1962) showed that 20 species isolated from estuarine sediments were not inhibited by salinity provided the temperature was maintained at 25°C and he also demonstrated that, at a range of temperatures, growth was never completely inhibited by salinity provided nutrients were available.

Gray, Pinto and Pathak (1963) conducted experiments that indicated that a number of fungi converted substrate carbon to tissue carbon most efficiently at the salinity of seawater and they attributed this to magnesium content, a suggestion which does not appear to have been followed up. Magnesium was available in significant amounts at both Crimdon and Newburn.

However, Borut and Johnson (1962) also demonstrated that an unknown constituent of seawater could inhibit germination of some fungal spores. The fungi grown from the beaches sampled in this work grew, sometimes in large numbers, on seawater based media and many of the colonies must have originated from spores; they did this at the ambient temperature of the beach and not at some artificially stabilised temperature generally higher than that ambient. It is therefore postulated that some species of the genera commonly isolated from these beaches can germinate and grow, even at low temperatures, at the salinity of seawater. It is probable too that they can sporulate given sufficient emersion time.

A generalised view must surely be that 'terrestial' (i.e. non-aquatic) fungi react to salinity as bacteria do to oxygen in that there are obligate halophiles and obligate halophobes with the majority of species being, to a greater or lesser degree, halotolerant.

The clear inverse relationship between bacterial numbers and those of the fungi which was revealed by the statistical analysis of the data is of great interest and, to my knowledge has not been previously re-

ported. The numbers of fungi grown from Zobell's medium were from cultures on which bacteria were also growing and there thus could have been some 'in vitro' antibiotic effect. However, further examination of the data showed that exclusion of the numbers of fungi grown on Zobell's did not alter the significant inverse relationship and conversely comparison of the numbers of bacteria and the numbers of fungal colonies grown on Zobell's showed no significant relationship.

It is possible, therefore, that there was 'in vivo' antibiosis between fungi and bacteria in the surface sand. Such antibiosis has usually been rejected as an ecological factor in the sea because of the very large dilution that must occur. The situation in the surface sand of the beach is, however, very different and it seems at least possible that antibiosis can occur.

It could, however, also be a coincident but different temperature effect. The higher temperatures, which seem to assist the growth of these fungi in saline conditions may simultaneously decrease, in some way, the number of bacteria or may occur when maximum sunlight is inhibiting multiplication of bacteria.

iii. GENERAL ECOLOGY

From the results obtained in this work and from a study of the literature an ecology of the sandy beach can be postulated:-

The littoral areas of the sea contain a significant amount of organic debris. Some of this detritus arises from the sea itself but the majority originates from the land; millions of dead leaves and other plant fragments being swept into the sea by every river and stream.

To this 'natural' detritus is added the very considerable contri-

bution from man and his activities, the majority of which is 'sewage'.

This organic matter is suspended in the seawater and behaves in a way that is different from that of dissolved substances. As Postma (1967) has shown, dissolved materials are transported from regions of high concentration to regions of low concentration whilst suspended matter behaves in the reverse manner and in many types of coastal environment is trapped and held near the shore. Much of it is, at some stage, caught in the breaker zone, where it is pounded by wave action and efficiently broken down into small particles. Because the minimum current velocity needed to resuspend this material, after deposition, is usually significantly greater than that required to keep it in motion after it has reached the bottom water (Postma, 1967) much of it is left on the beach when the tide recedes.

The beach provides an ideal substrate for the rapid production of bacteria since for its mass it has a huge surface area which, in the intertidal zone, as this work has shown, is constantly moist, and, as will be discussed later, such a situation provides the basis for optimum survival and multiplication.

Heterotrophic bacteria are thus available to colonise particles of organic matter and to convert its substance to soluble materials. It is possible that they are attracted to these particles by chemotactic means (see Bell and Mitchell, 1972) and it has been shown that the marine bacteria, which are predominantly present on a beach, do have the power to convert these organic materials (see e.g. Merkel, Braithwaite and Britzler, 1961; Merkel, Driesbach and Ziegler, 1975).

However, most bacteria do not have great penetrative power, therefore, the population on the surface of a particle will reach its limit when the surface-available nutrients are exhausted. A fresh tide, with its wave action will, however, free these particles to a large extent of their adsorbed bacteria and by pounding the material with the abrasive sand reveal new surfaces. As the tide recedes much of the newly

'homogenised' material will be redeposited and then recolonised by bacteria.

In this manner organic matter is continuously broken down and converted by heterotrophic bacteria to soluble substances which are transported, generally, out to sea. The beach, therefore, acts as a continuous conversion mechanism changing organic solids to soluble nutrients. In this way the bacteria are acting as the 'herbivores' (strictly detritivores) of the beach and also as the primary producers.

They are aided in this process by other organisms. The fungi of species (considered as terrestial) are deposited, from the air and from the seawater, onto the beach surface and some will adhere to organic particles. Some of the particles may already contain growing fungi. The spores of some species will germinate and their hyphae will penetrate the particles. This growth can continue whether the particle is lying on the beach or has been resuspended in seawater and the growing organism will utilise organic material, including some forms perhaps not available to bacteria. When these hyphae die new surfaces are again opened to bacterial action. The fungal mycelium itself may also be utilised as nutrients by some bacteria (Mitchell and Kirsen, 1968). Although these 'terrestial' fungi do not appear to have the power to sporulate in water it is probable that some do so whilst lying on or near the beach surface in the periods between tides. In any case aerial spores from a variety of sources provide a relatively constant new 'inoculum' for the beach. Gregory and Sreeramulu (1958) have shown that the air over estuaries may contain very large numbers of a variety of fungal spores.

Detritus feeding animals living in the beach also take part in the system. It has been shown repeatedly that the deposits on which they feed are consumed by many species in order to utilise either the bacteria which are adsorbed to the particles or their products present in the surface water film. (see e.g. Wilson, 1954, Crisp and Ryland, 1960, Meadows, 1964 and Gray, 1966).

The resultant faeces has been shown to contain most of the original organic material and this faeces will be reconsumed when recolonised by bacteria. These animals therefore have an effect similar to wave action in that they continually provide new surfaces for bacterial attack.

Sea birds feeding on the beach, in the surf zone or at sewage outfalls perhaps also act in a way similar to bacteria by consuming, in their case, the larger organic fragments and converting them to soluble products or smaller particles. Such products will commonly be deposited on the land but may also be transferred to the sea.

The quantitative aspects of this process e.g. bacterial numbers, carbon and nitrogen levels seem to be inversely related to particle size or, perhaps more accurately, directly related to total particle surface area. Whether it is the surface area of all particles or only the surface area of the organic portion which is important needs further investigation. To quote from Dale (1974) "the relationship between bacterial numbers, carbon and nitrogen cannot be satisfactorily explained until the nature of sedimentary organic matter and the dynamics of its use by microbes are closely examined. The existence of strong correlation suggests that such an examination would be worthwhile." Certainly the inorganic particles cannot be regarded as 'inactive' since they provide surfaces for adsorption of bacteria and nutrients but the surface of organic material open to bacterial attack must surely be a major factor in productivity. Hargrave (1972) calculated that there were three times more bacteria on the surface of organic particles than on the surface of inorganic particles. If this is so then a count of the percentage of organic particles is probably a more important investigation than measurement of carbon and nitrogen levels.

On the beach surface, in that area subject to wave action, other micro-flora and the micro-fauna probably do not play any great part. Wave action and sediment movement limit their growth (Steel and Baird, 1968). In more stable situations, e.g. in sublittoral sediments, they

probably have a much more important role to play and in the case of microfauna this role is one of repeated removal of bacteria from particle surfaces thus maintaining a bacterial population continually in the logarithmic phase (see Johannes, 1968). Along the litter line stranded seaweed and other organic matter has an ecology of its own. Bacteria which can break down the constituents of seaweed play a part but the role of the fungi which are present is uncertain (Chesters. et al.1966). Protozoan Ciliates are present in large numbers and probably feed on the bacterial population which may be very large (Rheinheimer, 1974).

iv. EFFECTS OF POLLUTION

The two beaches chosen for sampling both bordered a grossly polluted littoral zone. Newburn beach, however, was grossly and directly polluted and this was reflected in higher carbon and nitrogen levels and in increased counts of organic particle content. Although the manner in which the investigation was conducted makes the comparison of the beaches unacceptable from the statistical point of view there are some inferences which may be drawn from the data.

There was no gross, obvious difference in the bacterial populations of the two beaches either quantitatively or qualitatively. The fungal flora on the Newburn beach was consistently more diverse than that at Crimdon.

The Crimdon site always showed greater variation of results, both chemical and microbiological. This may have been inversely related to the degree of pollution; it could, however, have been related to the slope of the Crimdon site as compared to that of Newburn.

Attempts to isolate human faecal flora from the particulate deposit of both beaches gave consistently negative results. It should be noted, however, that no precautions were taken to secure the growth of 'stress-injured' coliform bacilli. The Medical Research Council (Report 1959) organised a national investigation of the risks to bathers on sewage polluted beaches. This was carried out using classical public health water examination techniques and was restricted to examination of the seawater; sediments were not examined. The results obtained showed that in a survey of ten beaches the median coliform count varied from 40 to 25 x 10^3/100 cm^3 of sea water. It is important to note that these are not counts of E. coli, though the committee directing the survey had satisfied themselves that there was sufficient correlation between the coliform and E. coli count in seawater (by carrying out the 'confirmatory' tests in some cases). The confirmatory tests did show that in some cases a high 'coliform' count was found to have a low 'E.coli' count but the committee believed that this 'occasional discrepancy' was of little practical importance. The overall conclusion of the report was that there was little risk to sea bathers arising from bathing in sewage polluted seawater.

Gerba and Keleod (1976) found that in laboratory experiments E. coli survived longer in seawater which contained sedimentary material and stated that these results explained why, on a volume basis, larger numbers of coliforms and faecal coliforms were found in estuarine sediments than in the overlying water, at field sites. Similarly Taylor, Nelson, Justice and Colwell (1975) found that in Chesapeake Bay 90% of the faecal indicator organisms were associated with suspended sediments and that faecal streptococci survived for prolonged periods in most of their sediment samples.

The difficulty of assessing the reports of large scale surveys of the effects of sewage is that the methods used are, of necessity, such that the 'faecal' organisms reported have rarely been specifically and

individually identified as Escherichia coli or Streptococcus faecalis. The single, simple method used in this work has failed to yield identifiable colonies of Escherichia coli but there is no doubt that a much more comprehensive investigation would be required to demonstrate the pattern of survival of human faecal bacteria on sandy beaches.

It has been possible to show that such bacteria do survive when present within the substance of a faecal stool. The R.S.C. report (1959) did point out that comminution of sewage was desirable on health grounds, as well as aesthetically, "to reduce the chances of contact with a heavy concentration of infective excreta from carriers (of disease) and to expose the disease-producing organisms to the disinfectant action of the seawater".

It is of interest to note that there have been reports of the isolation of Salmonella from the faeces of seagulls and although this has been associated with their habit of feeding on rubbish tips it could perhaps more logically be associated with feeding at sewage outfalls.

Sea coal was present on both these beaches both as drifts of larger particles and as finely particulate coal within the sand. Although the coal contributed little to the measured organic carbon levels this is in no way proof that its contribution as a nutrient, is equally small. Andrews, Floodgate and Pugh (1976) have demonstrated that many of the bacteria isolated from the beach are hydrocarbonoclastic and it is feasible, therefore, that finely particulate sea coal could be utilized. Similarly some of the genera of fungi isolated contain species which are known hydrocarbonoclasts.

v. IMPORTANCE OF ADSORPTION

As early as 1943 Zobell demonstrated convincingly that the presence of solid surfaces markedly increased the activity of marine bacteria. He showed that this was, at least partly due to the fact that such surfaces adsorbed nutrients. Up to 27% of the organic content of seawater could be adsorbed by a glass surface and the rate of attack on hydrocarbons by bacteria was accelerated 2 - 10 fold if those hydrocarbons were made available on the extensive surface of adsorbents such as sand.

He believed that the majority of bacteria in the sea were attached to particles and he demonstrated that there were definite differences in the attachment properties of different bacteria which were not related to Gram-staining properties.

He demonstrated two phases of adsorption - initial adsorption - when the organisms could be washed off and, secondary (occurring after a few hours) when they could not be removed by washing.

Using ordinary light microscopy he was able to observe, on glass slides from which the adherent bacteria had been removed, 'footprints', that is outlines of the bacteria in some faintly staining material which he believed was a secreted cementing substance.

Meadows (1964), removing bacteria from sand with various solutions, found that the supernatants contained numerous motile rods but Meadows and Anderson (1968) subsequently found that careful microscopical examination of sand grains revealed that the majority of attached bacteria were cocci some of which were embedded in a well-developed matrix which, in outline, appeared as a hump on the sand grain surface.

Marshall (1971) and Marshall, Stout and Mitchell (1971) confirmed Zobell's finding of two phases of bacterial adsorption and called the first 'reversible sorption' which they described as an essentially instantaneous attraction in which the bacteria are held weakly near the surface and preserve Brownian movement. They found that in this phase

the bacteria were readily removed by washing the surface with 2.5% sodium chloride. A second phase - irreversible sorption - was characterised by firm adhesion of the bacteria, no Brownian movement, and failure to wash off with 2.5% sodium chloride.

Using a non-motile strain of Achromobacter they found that reversible sorption took place and that this increased with increasing electrolyte concentration.

With a motile strain of Pseudomonas they showed that though irreversible sorption was negligible from sodium chloride solution it was considerable from artificial seawater. Glucose stimulated sorption from both media; calcium and magnesium seemed to be important and whilst extremely low levels of available carbon stimulated irreversible sorption higher levels inhibited this process.

Decreasing electrolyte concentrations resulted in an increase in 'repulsion energy' (desorption?) for both the reversible adsorption of the Achromobacter and the irreversible adsorption of the Pseudomonas.

Electron microscopy of attached bacteria showed formation of very fine, extracellular, polyrenic fibrils which presumably were equivalent to the cementing substance of Zobell since they too caused 'footprints'.

Stalked bacteria have been described by a number of workers when using Cholodny slides and Meadows and Anderson (1968) saw a few attached to sand grains. Some bacterial fimbriae are known to have attachment properties.

All these findings obviously, have profound significance in the study of the microbiology of marine sediments and explain many of the findings.

It is clear from my own experience of the difficulty of removing bacteria from sand (and that of other workers) that the majority of bacteria are 'irreversibly' adsorbed but it is probable that this situation could be radically changed if the interstitial water contained high levels of available nutrients. It is also clear that the removal

of bacteria by distilled water (= fresh water) demonstrated by Wagner and Schwartz (1961), by Meadows (1964) and confirmed in this work is due to decreasing electrolyte concentration or, possibly, dilution of nutrients or calcium and magnesium more specifically. It seems likely, therefore, that a heavy rainstorm could profoundly alter the bacterial population of a beach by soaking with fresh water from above and by increasing the capillary rise of fresh water from below.

My own second adsorption experiment shows that in normal circumstances about 60% of the bacteria present in seawater will be adsorbed when the seawater percolates through the upper layers of beach sand. The beach in these circumstances is behaving in the same way as the 'trickling filter' used in sewage purification and it is of interest that the bacterial flora present in the upper layers is similar to that of such a filter (see Higgins & Burns, 1975). My subsequent experiment demonstrated that the adsorption was a characteristic attributable to the organism and not to the particles, and this fits with the findings of Zobell (1943).

Zobell's findings on the adsorption of nutrients is also very important in the context of intertidal sediments. It could explain discrepancies between organic particle content and measured nitrogen and carbon levels. It could explain also the relationship between surface area and nutrient levels on those beaches with little particulate organic material and it tends to confirm the view of Steel and Baird (1968) that on such beaches the majority of organic nutrients is adsorbed to the sand. Wilson (1953) found that the majority of nitrogen could not be washed off intertidal sediment and assumed it must be present in insoluble form and 'organically bound'. Adsorption can thus explain how 'clean' beaches can retain sufficient nutrient material to maintain a microbial population in spite of diurnal inundation.

It is apparent from the tide imitation experiment that the pounding of the waves is sufficient to remove even the 'irreversibly' adsorbed

bacteria from the surface sand and since there was no evidence of re-adsorption the majority of these bacteria must be swept, in suspension, into the littoral zone. If this is true then the population must be substantially reduced and the beach must, therefore, have a high productivity to maintain its bacterial population levels.

The final adsorption experiment indicated that, on this single sampling occasion, 75% of the bacteria were 'irreversibly' adsorbed to the sand particles since only 25% were removed by washing. However, as already pointed out, the relationship between adsorbed bacteria and those either reversibly adsorbed or free in the interstitial water is almost certainly not a constant and may depend on nutrient concentration in the interstitial water.

It is very probable that adsorption is also important in the ecology of fungi on the sandy shore although there has been no experimental work to demonstrate that this is so, to my knowledge.

The most significant finding has been that of Burges (1950) who had noticed that there seemed to be some vertical zonation of fungi in sandy soil. He, therefore, tested the retention of spores from three genera - Penicillium, Zygorrhynchus and Gliomastix - by columns of sand. He found that Zygorrhynchus and Gliomastix were washed readily through the column by fresh water, whereas the Penicillium spores showed very little movement. He attributed this difference to the fact that the spores of Penicillium have a waxy, non-wetting coat whilst the others had a mucilagenous, wettable coat.

Although no formal experiment has been carried out to test adsorption of spores of fungi it has been noted that fresh seawater yielded a moderate number of colonies of fungi whilst the same seawater having been passed through a 10 cm sand column yielded none.

Direct observation of the sand grains showed a few hyphal fragments and these appeared to be firmly attached to the surface of the grain. Brown's comments (1958a) that the failure of her direct counts by the

Jones and Mollison method were due to the adherence of hyphae to the coarse soil fractions also suggests that the adhesion of hyphae to sand is important.

The phenomenon of adsorption and adhesion is obviously a complex one. Anderson and Meadows (1969) suggested that 'zeta potential' was an explanation for adsorption and Marshall, Stout and Mitchell (1971) also mention electrostatic effects. This suggestion would accord with the apparent connection with electrolyte concentration and the apparent importance of calcium and magnesium which are known to be important factors in 'zeta potential'. The attractive forces of van der Waal are known to be affected by many factors and the subject is so complex that it forms a whole branch of science on its own. Further suggestion that electrostatic forces play some part is the finding of both Anderson and Meadows (1969) and Marshall, Stout and Mitchell (1971) that bacteria removed from surfaces, after an interval aggregated together. This was not observed to occur with the bacteria suspended in natural seawater in the tide - imitation experiment carried out by me.

H. FURTHER WORK REQUIRED

The investigation of the microbial ecology of sandy beaches is still to a large extent in the observational stage and much more data are required before definitive experiments can be designed and carried out. However, Andrews, Floodgate and Pugh (1976) have made a valuable start with their work on model beaches and the work carried out for this thesis when viewed in the context of the reports of other workers reveals some practical steps that will help to elucidate the problems.

The utilisation of nutrients by both bacteria and fungi needs to be examined in detail in order to explain the apparent relationship between the level of organic material, the surface area of particles and the numbers of bacteria. The inverse relationship between bacterial and fungal numbers suggested by my data needs to be confirmed and explained. The life cycle of fungi in intertidal substrates needs to be followed and a model beach would provide an excellent substrate to do this. It needs to be shown whether the fungi isolated from the sand can and do sporulate between tides.

The effects of pollution by sewage needs to be thoroughly investigated to show the pattern of survival of human faecal organisms in this substrate and to confirm the suggestion that such pollution does not materially alter the levels of bacteria present, perhaps because of the repeated effects of desorption by wave action. The possibility that sea coal may act as a nutrient source for beach bacteria needs to be examined.

The phenomenon of adsorption both of bacteria and fungi needs examination in depth - its effects on zonation; changes of flora after heavy rain, changes that could be caused by pollution by detergents and many other ecological aspects will depend upon a clarification of means by which micro-organisms adhere to particles.

All such work would have implications for the ecology of marine

sediments generally and thus could provide, from a readily available substrate, information of wide-ranging importance.

I. C O N C L U S I O N S

This work has shown that there was a diverse resident population of heterotrophic bacteria on the sandy beaches examined. The majority of these were gram negative rods and most of them could be placed into the Pseudomonas genus. Flavobacterium and Corynobacterium species were also common.

There were also fungi present and most of those grown by the methods employed were from genera normally described as 'torrential'. These fungi could be isolated at ambient beach temperature on media which contained only those nutrients that would normally be present on a beach, and which had a seawater base, indicating the ability of these species to germinate and grow in these conditions.

Statistical analysis of the data indicated that numbers of bacteria were steady throughout the year with a possible reduction in numbers in the hotter summer months. The number of bacteria varied significantly, however, from a spatial point of view.

Number of fungi grown was, in contrast, comparatively uniform spatially and the data suggested an increase in numbers with higher temperatures. Correlation of bacterial and fungal data showed an inverse relationship.

Sewage contamination of one of the beaches apparently resulted in only a relatively small increase in organic nutrient levels and there was no evidence of a consequent increase in the numbers of bacteria or fungi. There was, however, a consistently more diverse fungal flora in the presence of direct sewage contamination.

Attempts to recover Escherichia coli from the beaches were not successful.

It is postulated that the phenomenon of adsorption is of profound ecological significance on these beaches and that it is unlikely that desiccation plays a significant part in modifying the flora of the

intertidal zone.

J. APPENDIX I

BACTERIAL COUNTS

All counts expressed as x $10^6/g$ of wet sand. (S.T. Sand temperature when sampled; T.E. Extremes of temperature to which cultures exposed; M.D.T. Mean of daily temperature recordings during incubation).

HEBBURN SITE

23.11.75	S.T. 5.9		T.E. 0.1 - 9.4	M.D.T. 4.9	
Sample	1	2	3	4	5
	0.98	5.80	6.50	4.82	3.18
	3.88	3.44	4.44	5.36	5.74
	4.34	2.08	5.34	3.34	4.16
	3.86	3.00	5.06	6.40	5.32
	3.70	5.20	4.52	5.90	5.38
	5.28	1.74	3.40	5.66	7.34
	5.36	4.08	3.38	4.96	8.52
	6.36	4.16	4.52	5.74	(20.00)
	4.34	3.96	4.96	3.32	O.G.
	3.32	2.94	5.00	6.66	O.G.
Mean	4.14	3.64	4.71	5.22	5.66

Overall mean 4.67 Sample range: 3.64 - 5.66; Plate range: 0.98-8.52

31.10.76	S.T. 4.8		T.E. 0.7 - 9.6	M.D.T. 4.6	
Sample	1	2	3	4	5
	3.96	1.98	2.84	2.52	3.96
	1.80	2.06	4.30	2.80	3.52
	2.94	1.52	2.80	2.20	4.04
	2.64	2.40	3.40	1.50	3.80
	2.86	1.60	3.70	2.60	3.28
	2.40	2.24	2.60	2.92	4.52
	3.64	1.90	4.20	3.44	4.20
Mean	2.89	1.96	3.40	2.57	3.90

Overall mean 2.94 Sample range: 1.96 - 3.90; Plate range: 1.50-4.52

7.11.76	S.T. 4.0	T.S. 0.7 - 8.2		N.D.T. 4.5	
Sample	1	2	3	4	5
	6.40	19.60	1.74	1.02	1.60
	9.00	6.12	0.98	1.52	1.78
	2.48	2.84	1.04	1.36	1.90
	4.48	10.04	1.42	1.18	2.02
	3.36	4.80	1.16	1.34	1.56
	4.20	3.26	1.80	1.72	1.98
	3.68	5.12	1.42	1.20	1.66
Mean	4.80	7.40	1.37	1.33	1.79

Overall mean: 3.34 Sample range: 1.33-7.40; Plate range: 0.98-19.60

CRIMDON SITE

11.5.76	S.T. 8.0	T.S. 9.4 - 15.5		N.D.T. 12.1	
Sample	1	2	3	4	5
	11.20	9.36	1.14	(20.00	1.58
	9.00	8.16	1.56	>20.00	2.30
	12.08	6.48	1.14	>20.00	2.40
	8.32	7.42	1.04	>20.00	1.98
	16.56	6.68	1.48	>20.00	1.46
	12.96	8.24	1.16	>20.00	2.60
	12.96	8.20	1.02	>20.00)	1.80
Mean	11.87	7.79	1.12		2.02

Overall mean: 5.70 Sample range: 1.12-11.87; Plate range: 1.02-16.56

11.8.76	S.T. 14.7	T.E. 15.0 - 20.0		H.D.T. 17.3	
Sample	1	2	3	4	5
	2.42	0.58	0.30	0.32	0.28
	3.20	0.36	0.20	0.78	0.40
	3.76	0.56	0.52	0.48	0.18
	5.36	0.66	0.46	0.46	0.38
	4.24	0.46	0.40	0.60	0.44
	2.60	0.60	0.30	0.66	0.40
	3.56	0.56	0.54	0.66	0.66
Mean	3.59	0.54	0.39	0.56	0.39

Overall mean: 1.09 Sample range: 0.39-3.59; Plate range: 0.18-5.36

13.3.77	S.T. 5.5	T.E. 2.2 - 9.4		H.D.T. 6.5	
Sample	1	2	3	4	5
	2.46	6.42	1.05	3.36	4.64
	3.20	7.10	1.36	3.32	4.22
	2.64	2.32	1.78	2.46	4.10
	3.58	3.24	2.30	3.02	3.01
	4.62	1.08	2.24	2.64	4.58
	3.46	2.72	0.96	3.80	2.82
	2.10	4.36	1.84	2.22	3.56
Mean	3.15	3.89	1.64	2.97	3.85

Overall mean: 3.10 Sample range: 1.65 - 3.89; Plate range: 0.96-7.10

APPENDIX II

FUNGAL COUNTS

All figures represent the total number of colonies growing on 7 plates of each medium.

NEWBURN SITE

23.11.75	S.T. 5.9	T.E. 0.1 - 9.4				N.D.T. 4.6
	1	2	3	4	5	Total for Medium
Zobell's	2	3	12	7	5	29
Corn Meal	39	8	5	0	5	57
Seawater	32	1	2	2	6	43
Seaweed	8	2	7	5	12	34
Total for sample	81	14	26	14	28	

Mean:- 33 (equivalent to 1178 colony forming units/g wet sand).

Sample range: 13 - 81

31.10.76	S.T. 4.8	T.E. 0.7 - 9.6				N.D.T. 5.6
	1	2	3	4	5	Total for Medium
Zobell's	22	6	27	35	54	144
Corn Meal	7	41	21	5	31	105
Seawater	13	37	131	4	24	209
Seaweed	13	25	30	16	13	97
Total for sample	55	109	209	60	122	

Mean:- 111 (equivalent to 3963 colony forming units/g wet sand).

Sample range: 55 - 209

7.11.76	S.T. 4.0	T.E. 0.0 - 9.8			M.D.T. 4.4	
	1	2	3	4	5	Total for Medium
Zobell's	24	15	5	1	2	47
Corn Meal	4	14	32	34	10	94
Seawater	4	3	29	112	61	209
Seaweed	1	13	128	17	28	187
Total for sample	33	45	194	164	101	

Mean: 107 (equivalent to 3819 colony forming units/g wet sand)

Sample range: 33 - 194

CRIMDON SITE

11.5.76	S.T. 8.0	T.E. 9.2 - 18.4			M.D.T. 12.5	
	1	2	3	4	5	Total for Medium
Zobell's	9	3	20	4	3	39
Corn Meal	46	26	5	157	77	311
Seawater	1	0	2	3	0	6
Seaweed	0	2	4	82	1	89
Total for sample	56	31	31	246	81	

Mean: 89 (equivalent to 3177 colony forming units/g wet sand)

Sample range: 31 - 246

11.8.76	S.T. 14.7	T.E. 11.1 - 20.0			H.D.T. 16.4	
	1	2	3	4	5	Total for Medium
Kobell's	77	28	12	11	19	147
Corn Meal	825	500	51	128	68	1572
Seawater	688	646	91	131	67	1623
Seaweed	359	309	86	116	72	942
Total for sample	1949	1483	240	386	226	

Mean: 857 (equivalent to 30595 colony forming units/g wet sand)

Sample range: 226 - 1949

13.3.77	S.T. 3.7	T.E. 1.7 - 9.4			H.D.T. 3.7	
	1	2	3	4	5	Total for Medium
Kobell's	12	5	6	3	10	36
Corn Meal	82	110	35	20	76	323
Seawater	148	272	92	112	300	924
Seaweed	71	68	32	81	210	462
Total for sample	313	455	165	216	596	

Mean: 349 (equivalent to 12459 colony forming units/g wet sand)

Sample range: 168 - 596

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